



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>A61K 39/395, 39/42, C12Q 1/00, 1/70, G01N 33/53</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/69462</b> <b>(43) International Publication Date:</b> 23 November 2000 (23.11.00)
<p><b>(21) International Application Number:</b> PCT/US00/13694</p> <p><b>(22) International Filing Date:</b> 18 May 2000 (18.05.00)</p> <p><b>(30) Priority Data:</b>          60/134,702                      18 May 1999 (18.05.99)                      US</p> <p><b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).</p> <p><b>(72) Inventors; and</b>  <b>(75) Inventors/Applicants (for US only):</b> GROSS, Mitchell, S. [US/US]; 667 Pugh Road, Wayne, PA 19087 (US). SWEET, Raymond, W. [US/US]; 108 Edgehill Road, Bala Cynwyd, PA 19004 (US). TAYLOR, Geraldine [GB/GB]; Compton, Newbury, Berkshire RG20 7NN (GB).</p> <p><b>(74) Agents:</b> BAUMEISTER, Kirk et al.; Smithkline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).</p>		<p><b>(81) Designated States:</b> AE, AL, AU, BA, BB, BG, BR, CA, CN, CZ, DZ, EE, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, TZ, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.</i></p>
<p><b>(54) Title:</b> HUMAN MONOCLONAL ANTIBODY</p> <p><b>(57) Abstract</b></p> <p>This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV). Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.</p>		

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## HUMAN MONOCLONAL ANTIBODY

**Field of the Invention**

5           This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV).  
10   Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.

**Background of the Invention**

15           Respiratory syncytial virus (RSV) is the major cause of lower respiratory disease in children, giving rise to predictable annual epidemics of bronchiolitis and pneumonia in children worldwide. The virus is highly contagious, and infections can occur at any age.  
20   Comprehensive details concerning RSV infection and its clinical features can be obtained from excellent recent reviews by McIntosh, K. and R. M. Chanock, In: "Respiratory Syncytial Virus", Ch. 38, B.N. Fields ed., Raven Press (1990) and Hall, C.B., In: "Textbook of  
25   Pediatric Disease" Feigin and Cherry, eds., W.B. Saunders, pgs 1247-1268 (1987).

          RSV is distributed worldwide. One of the most remarkable features of the epidemiology of RSV virus, as mentioned above, is the consistent pattern of infection  
30   and disease. Other respiratory viruses cause epidemics at irregular intervals or exhibit a mixed endemic/epidemic pattern, but RSV is the only respiratory viral pathogen that produces a sizable epidemic every year in large urban centers. In the

temperate areas of the world, RSV epidemics have occurred primarily in the late fall, winter or spring but never during the summer. The occurrence and spread of infection within a community is characteristic and easily diagnosed, leading to sharp rises in cases of bronchiolitis and pediatric pneumonia and the number of hospital admissions of young children with acute lower respiratory tract disease. Other respiratory viral agents that occur in outbreaks are rarely present at the same time as RSV.

Primary RSV infection occurs in the very young. Zero to 2 year old infants are the most susceptible and represent the primary affected population. In this group, 1 out of 5 will develop lower respiratory (below larynx) disease upon infection and this ratio stays the same upon reinfection. By 1 year of age, 25-50% of infants have specific antibodies as a result of natural infection and this is close to 100% by age 4-5. Thus, virtually all children have been infected before they have entered school.

Age, sex, socioeconomic and environmental factors can all influence the severity of disease. Hospitalization is required in 1-3% of cases of RSV infection and is usually of long duration (up to 3 weeks). The high morbidity of RSV infection, especially in infancy, has also been implicated in the development of respiratory problems later in life. With current intensive care in the U.S. and the other developed countries, overall mortality for normal subjects is low (less than 2% of hospitalized subjects). However, mortality is much higher in less developed countries and, even in developed countries, mortality is high in certain risk groups such as in infants with underlying cardiac condition (cyanotic congenital heart disease) or

respiratory disease (bronchopulmonary dysplasia) where the progression of symptoms may be rapid. For instance, mortality in infants with cyanotic congenital heart disease has been reported to be as high as 37%. In  
5 premature infants apneic spells due to RSV infection may occur and, in rare cases, cause neurologic or systemic damage. Severe lower respiratory tract illness (bronchiolitis and pneumonia) is most common in patients under six months of age. Infants who have apparently  
10 recovered completely from this illness may display symptomatic respiratory abnormalities for years (recurrent wheezing, decreased pulmonary function, recurrent cough, asthma, and bronchitis).

Immunity to RSV appears to be short-lived, thus  
15 reinfections are frequent. The mechanisms by which the immune system protects against RSV infection and reinfection are not well understood. It is clear, however, that immunity is only partially protective since reinfection is common at all ages, and sometimes  
20 occurs in infants only weeks after recovery from a primary infection. Both serum and secretory antibodies (IgA) have been detected in response to RSV infection in adults as well as in very young infants. However, the titers of serum antibodies to the viral F or G  
25 glycoprotein, as well as of neutralizing antibodies found in infants (1-8 months of age) are 15-25% of those found in older subjects. These reduced titers may contribute to the increased incidence of serious infection in younger children.

30 Evidence for the role of serum antibodies in protection against RSV virus has emerged from epidemiological as well as animal studies. In adults exposed naturally to the virus, susceptibility correlated well with low serum antibody level. In

infants, titers of maternally transmitted antibodies correlate with resistance to serious disease [Glezen, W.P. et al., J. Pediatr. 98:708-715 (1981)]. Other studies show that the incidence and severity of lower respiratory tract involvement is diminished in the presence of high serum antibody [McIntosh, K. et al., J. Infect. Dis. 138:24-32 (1978)] and high titers of passively administered serum neutralizing antibodies have been shown to be protective in a cotton rat model of RSV infection [Prince, G. A. et al., Virus Res. 3:193-206 (1985)].

Children lacking cell-mediated immunity are unable to overcome their infection and shed virus for many months in contrast to children with normal immune systems. Similarly, nude mice infected with RSV virus persistently shed virus. These mice can be cured by adoptive transfer of primed T cells [Cannon, M. J. et al., Immunology 62:133-138 (1987)].

In summary, it appears that both cellular and humoral immunity are involved in protection against infection, reinfection and RSV disease and that although antigenic variation is limited, protective immunity is not complete even after multiple exposures.

RSV, belonging to the family paramyxoviridae, is a negative-strand unsegmented RNA virus with properties similar to those of the paramyxoviruses. It has, however been placed in a separate genus Pneumovirus, based on morphologic differences and lack of hemagglutinin and neuraminidase activities. RSV is pleomorphic and ranges in size from 150-300 nm in diameter. The virus matures by budding from the outer membrane of a cell and virions appear as membrane-bound particles with short, closely spaced projections or "spikes". The RNA genome encodes 10 unique viral

polypeptides ranging in size from 9.5 kDa to 160 kDa [Huang, Y. T. and G. W. Wertz, J. Virol. 43:150-157 (1982)]. Seven proteins (F, G, N, P, L, M, M2) are present in RSV virions and at least three proteins (F, G, and SH) are expressed on the surface of infected cells. The F protein [SEQ ID NO: 20] has been conclusively identified as the protein responsible for cell fusion since specific antibodies to this protein inhibit syncytia formation *in vitro* and cells infected with vaccinia virus expressing recombinant F protein form syncytia in the absence of other RSV virus proteins. In contrast, antibodies to the G protein do not block syncytia formation but prevent attachment of the virus to cells.

RSV can be divided into two antigenically distinct subgroups, (A & B) [Mufson, M. A. et al., J. Gen'l. Virol. 66:2111-2124 (1985)]. This antigenic dimorphism is linked primarily to the surface attachment (G) glycoprotein [Johnson, R. A. et al., Proc. Nat'l. Acad. Sci. USA 84:5625-5629 (1987)]. Strains of both group A and B circulate simultaneously, but the proportion of each may vary unpredictably from year to year. An effective therapy must therefore target both subgroups of the virus and this is the reason for the selection of the highly conserved surface fusion (F) protein as target antigen for mAb therapy as will be discussed later.

The induction of neutralizing antibodies to RSV virus appears to be limited to the F and G surface glycoproteins. Of these two proteins, the F protein is the major target for cross-reactive neutralizing antibodies associated with protection against different strains of RSV virus. In addition, experimental vaccination of mice or cotton rats with F protein also

results in cross protection. The antigenic relatedness of the F protein across strains and subgroups of the virus is reflected in its high degree of homology at the amino acid level. In contrast, in the two subgroups and various strains of RSV, antigenic dimorphism was linked primarily to the G glycoprotein. The F protein has a predicted molecular weight of 68-70 kDa; a signal peptide at its N-terminus; a membrane anchor domain at its C terminus; and is cleaved proteolytically in the infected cell prior to virion assembly to yield disulfide linked F<sub>2</sub> and F<sub>1</sub>. Five neutralizing epitopes have been identified within the F protein sequence [SEQ ID NO: 20] and map to residues 205-225; 259-278; 289-299; 483-488 and 417-438. Studies to determine the frequency of sequence diversion in the F protein [SEQ ID NO: 20] showed that the majority of the neutralizing epitopes were conserved in all of the 23 strains of RSV virus isolated in Australia, Europe, and regions of the U.S. over a period of thirty years. In another study, seroresponses of forty three infants and young children to primary infection with subgroup A or a subgroup B strain showed that responses to homologous and heterologous F antigens were not significantly different, while the G proteins of the subgroup A and B strains were quite unrelated. Moreover, antibody inhibition of virus-mediated cell fusion *in vitro* versus inhibition of infection correlates best with protection in animal models and fusion inhibition is primarily restricted to F protein specific antibodies.

Prophylactic treatment for RSV infection is thus desirable for the high risk groups of children as well as for all children in underdeveloped countries. However, a vaccine for RSV infection is not currently available. Severe safety issues surrounding an

attenuated whole virus vaccine tested in the 1960s, as well as the potential of induced immunopathology associated with the newer candidate subunit vaccines make the prospects of a vaccine in the near future appear remote. To date one drug therapy, Ribavirin, a broad spectrum antiviral, has been approved. Ribavirin has gained only minimal acceptance owing to problems of administration, mild toxicity and questionable efficacy. In the majority of cases, hospitalized children receive no drug therapy and receive only intensive supportive care which is extremely costly. It is clear that there is a need for a safe, effective and easily administered drug for the treatment of RSV infection.

The use of passive antibody therapy in humans is well documented and is being used to treat other infectious diseases such as hepatitis and cytomegalovirus. The feasibility of passive antibody treatment/protection against RSV has been well established using animal models. Most of the earlier passive transfer studies in animals against infectious agents, including RSV, utilized murine mABs. Studies in animals have clearly demonstrated that polyclonal and monoclonal antibody against both F and G glycoprotein can confer passive protection in RSV virus infection when given prophylactically or therapeutically [Prince, *et al.*, supra]. In these studies, passive transfer of neutralizing F or G mABs to mice, cotton rats or monkeys, significantly reduce or completely prevent replication of the RSV virus in the lungs. However, as discussed above, clearly, the F protein is the more important target for antibody therapy.

Recently, the FDA has approved for use intravenous gammaglobulins (IVIG) isolated from pooled human sera. Initial reports from this study had been encouraging

[Groothuis, J. R. et al., Antimicrob. Agents Chemo. 35(7):1469-1473 (1991)]. However, generic shortcomings of IVIGs exist and include, without limitation, the fact that such products are human blood derived and grams of antibody often need to be administered to achieve an effective dose.

Alternatively, monoclonal antibodies have been employed. The advantages of such an approach include: a higher concentration of specific antibody can be achieved thereby reducing the amount of globulin required to be given; the reliance on direct blood products can be eliminated; the levels of antibody in the preparation can be more uniformly controlled and the routes of administration can be extended. While passive immunotherapy employing monoclonal antibodies from a heterologous species (e.g., murine) has been suggested (See: PCT Application PCT/US94/08699, Publication No. WO 95/04081), one alternative to reduce the risk of an undesirable immune response on the part of the patient directed against the foreign antibody is to employ "humanized" antibodies. These antibodies are substantially of human origin, with only the Complementarity Determining Regions (CDRs) being of non-human origin. Particularly useful examples of this approach are disclosed in PCT Application PCT/GB91/01554, Publication No. WO 92/04381 and PCT Application PCT/GB93/00725, Publication No. WO93/20210. Clinical trials are on-going to evaluate the efficacy of humanized antibodies for treatment of RSV infection in young children.

A second and more preferred approach is to employ fully human mAbs. Unfortunately, there have been few successes in producing human monoclonal antibodies through classic hybridoma technology. Indeed,

acceptable human fusion partners have not been identified and murine myeloma fusion partners do not work well with human cells, yielding unstable and low producing hybridoma lines. However, recent advances in molecular biology and immunology make it now possible to isolate human mABs, particularly directed against foreign infectious agents.

Fully human mAbs to RSV F protein [SEQ ID NO: 20] remain a desirable option for the treatment of this disease. Although some success has been reported in obtaining fragments of such mAbs [Barbas, C.F. et al., Proc. Nat'l. Acad. Sci. USA 89:10164-10168 (1992); Crowe, J. E. et al., Proc. Nat'l. Acad. Sci. USA 91:1386-1390 (1994) and PCT application number PCT/US93/08786, published as WO94/06448, March 31, 1994)], the achievement of such results is not straightforward. Novel human mABs, when and however obtained, are particularly useful alone or in combination with existing molecules to form immunotherapeutic compositions.

There exists a need in the art for useful prophylactic compositions for the prevention or passive treatment of RSV.

## **Brief Description of the Invention**

In one aspect, this invention provides fully human monoclonal antibodies and functional fragments thereof specifically reactive with an F protein epitope of RSV and capable of neutralizing RSV infection. These human mABs specific for the F protein of RSV virus may be useful to passively treat or prevent infection.

In another aspect, the present invention provides modifications to neutralizing single chain Fv fragments (scFV) specific for the F protein of RSV produced by

random combinatorial cloning of human antibody sequences and isolated from a filamentous phage Fab display library.

5 In still another aspect, there is provided a reshaped or altered human antibody containing human heavy and light chain constant regions from a first human donor and heavy and light chain variable regions or the CDRs thereof derived from human neutralizing monoclonal antibodies for the F protein of RSV derived  
10 from a second human donor.

In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) altered or reshaped antibodies and a pharmaceutically acceptable carrier.

15 In yet another aspect, the invention provides a pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the reshaped, altered or monoclonal antibody of this invention in combination with at least one additional  
20 monoclonal, altered or reshaped antibody. A particular embodiment is provided in which the additional antibody is an anti-RSV antibody distinguished from the subject antibody of the invention by virtue of being reactive with a different epitope of the RSV F protein antigen  
25 than the subject antibody of the invention.

In a further aspect, the present invention provides a method for passive immunotherapy of RSV disease in a human by administering to said human an effective amount of the pharmaceutical composition of the invention for  
30 the prophylactic or therapeutic treatment of RSV infection.

In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of human and altered antibodies

(e.g., engineered antibodies, CDRs, Fab or F(ab)<sub>2</sub> fragments, or analogs thereof) which are derived from human neutralizing monoclonal antibodies (mAbs) for the F protein of RSV. These components include isolated  
5 nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the  
10 recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that the human or altered antibody is expressed in said cells and isolating the expressed product therefrom.

15 In still another aspect of the invention is a method to diagnose the presence of RSV in a human which comprises contacting a sample of biological fluid with the human antibodies and altered antibodies and fragments thereof of the instant invention and assaying  
20 for the occurrence of binding between said human antibody (or altered antibody, or fragment) and RSV.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

25

#### **Brief Description of the Drawings**

Fig. 1A is a graph illustrating the competition of G $\lambda$ -1 scFV phage binding with RSV19 mAb [International patent publication No. WO92/04381, published March 19,  
30 1992].

Fig. 1B is a graph illustrating the competition of G $\lambda$ -1 scFV phage binding with RSV B4 mAb [International patent publication No. WO93/20210, published October 14, 1993].

Fig. 2 is a graph illustrating virus neutralization by scFV phages, G $\lambda$ -1, G $\lambda$ -3, and G $\kappa$ -1 with RSV strain 273.

Fig. 3 illustrates the DNA sequence [SEQ ID NO: 1] and protein sequence (amino acids reported in single letter code) [SEQ ID NO: 2] for the G $\lambda$ -1 light chain variable region, processed N-terminus through framework IV.

Fig. 4 illustrates the DNA sequence [SEQ ID NO: 3] and protein sequence (amino acids reported in single letter code) [SEQ ID NO: 4] for the G $\lambda$ -1 heavy chain variable region, processed N-terminus through framework IV.

Fig. 5 illustrates the cloning strategy used for the construction of the G $\lambda$ -1 monoclonal antibody. The heavy chain V region was cloned into the pCD derivative vector as a XhoI - ApaI fragment. The entire light chain V region was cloned into the pCN derivative vector, 43-1pcn, as a SacI - AvrII fragment. Details are described below.

Fig. 6 provides a comparison of the heavy chain amino acid sequences of the G $\lambda$ -1 single chain F<sub>v</sub> [SEQ ID NO: 5] and various monoclonal antibodies of this invention. The amino acid sequences of the heavy chains for the A [SEQ ID NO: 7] and B [SEQ ID NO: 8] constructs are shown. Numbering of the residues is based on the germline (GL) gene Dp58 [SEQ ID NO: 6], beginning at the mature processed amino terminus and ending at CDR3. The "-" indicates identity to the preceding sequence (eg., A compared to B). Bold residues correspond to the leader region, and to CDRs 1-3.

Fig. 7 provides a comparison of the light chain amino acid sequences of the G $\lambda$ -1A single chain F<sub>v</sub> [SEQ ID NO: 9] and various monoclonal antibodies of this

invention. The amino acid sequences of the light chains for the A [SEQ ID NO: 11] and B [SEQ ID NO: 12] constructs are shown. Numbering of the residues in the VK region is based on the germline (GL) gene DpL8 [SEQ ID NO: 10], beginning at the mature processed amino terminus and ending at CDR3. For reference to framework 4, the actual numbering is also shown for G $\lambda$ -1A. As in Fig. 6, the "-" indicates identity to the preceding sequence.

10 Figs. 8A to 8F illustrate the continuous DNA sequence [SEQ ID NO: 13] of the expression plasmid G $\lambda$ -1Apcd containing the RSV neutralizing human G $\lambda$ -1 mAb for the heavy chain. The start of translation, leader peptide, amino-terminal processing site, carboxy  
15 terminus of the G $\lambda$ -1 heavy chain, and *Eco* RI restriction endonuclease cleavage site are shown.

Figs. 9A to 9E illustrate the continuous DNA sequence [SEQ ID NO: 14] of the expression plasmid G $\lambda$ -1Apcn containing the RSV neutralizing human G $\lambda$ -1 mAb for  
20 the light chain. The corresponding features for the light chain as for Figs. 8A-8F are shown.

Figs. 10A and 10B illustrate the continuous DNA sequence [SEQ ID NO: 15] of the coding region of the heavy chain of plasmid G $\lambda$ -1Bpcd. Bolded residues  
25 indicate differences from the full vector sequence for G $\lambda$ -1Apcd in Figs. 8A-8F [SEQ ID NO: 13].

Fig. 11 is the DNA sequence [SEQ ID NO: 16] of the coding region for the light chain of plasmid G $\lambda$ -1Bpcn. Bolded residues indicate differences from the full  
30 vector sequence for G $\lambda$ -1Apcn in Figs. 9A-9E [SEQ ID NO: 14].

**Detailed Description of the Invention**

This invention provides useful human monoclonal antibodies (and fragments thereof) reactive with the F protein of RSV, isolated nucleic acids encoding same and various means for their recombinant production as well as therapeutic, prophylactic and diagnostic uses of such antibodies and fragments thereof.

*I. Definitions.*

As used in this specification and the claims, the following terms are defined as follows:

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric, humanized, or reshaped or immunologically edited human antibodies) or fragments thereof lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab')<sub>2</sub> and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding an altered antibody of the invention or a fragment thereof.

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferably all six CDRs are replaced. More preferably an entire antigen combining region (e.g., Fv, Fab or F(ab')<sub>2</sub>) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a full length monoclonal antibody.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor human antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example, Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second fusion partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably the fusion partner is an immunoglobulin gene and when so, it is referred to as a "second immunoglobulin partner". The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found

in a Fab, or F(ab)<sub>2</sub> (i.e., a discrete part of an appropriate human constant region or framework region).

A second fusion partner may also comprise a sequence encoding an integral membrane protein exposed on the  
5 outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase (HRP),  $\beta$ -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab')<sub>2</sub> are used with  
10 their standard meanings [see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)].

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length  
15 synthetic antibody (e.g., a chimeric, humanized, reshaped or immunologically edited human antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous  
20 parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa.  
25 Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise  
30 replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring

variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody from a heterologous species.

5       A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In  
10 addition, framework support residues may be altered to preserve binding affinity [see, e.g., Queen *et al.*, Proc. Nat'l. Acad. Sci. USA, 86:10029-10032 (1989), Hodgson *et al.*, Bio/Technology, 9:421 (1991)].

      An "immunologically edited antibody" refers to a  
15 type of engineered antibody in which changes are made in donor and/or acceptor sequences to edit regions in respect of cloning artifacts, germ line enhancements, etc. aimed at reducing the likelihood of an immunological response to the antibody on the part of a  
20 patient being treated with the edited antibody.

      The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first  
25 immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this  
30 invention is a Fab fragment of a human neutralizing monoclonal antibody designated as Fab G $\lambda$ -1. Fab G $\lambda$ -1 is defined as a having the variable light and heavy chain DNA and amino acid sequences G $\lambda$ -1 as shown in Figs. 3, 4, 8A-8F and 9A-9E [SEQ ID NOS: 1-4, 13 and 14].

The term "acceptor antibody" refers to an antibody (monoclonal or recombinant) from a source genetically unrelated to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains [see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)]. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By "sharing the antigen binding specificity or neutralizing ability" is meant, for example, that although Fab G $\lambda$ -1 may be characterized by a certain level of antigen affinity, a CDR encoded by a nucleic acid sequence of Fab G $\lambda$ -1 in an appropriate structural environment may have a lower, or higher affinity. It is

expected that CDRs of Fab G $\lambda$ -1 in such environments will nevertheless recognize the same epitope(s) as does the intact Fab G $\lambda$ -1. A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor  
5 deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by  
10 at least one amino acid, wherein said modification can be a chemical modification, or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen  
15 specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

20 Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic  
25 code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein  
30 carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used

in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore (Pharmacia) system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

10        *II. Combinatorial Cloning.*

As mentioned above, a number of problems have hampered the direct application of the hybridoma technology [G. Kohler and C. Milstein, Nature, 256: 495-497 (1975)] to the generation and isolation of human monoclonal antibodies. Among these are a lack of suitable fusion partner myeloma cell lines used to form hybridoma cell lines as well as the poor stability of such hybridomas even when formed. These shortcomings are further exacerbated in the case of RSV because of the paucity of viral specific B cells in the peripheral circulation. Therefore, the molecular biological approach of combinatorial cloning is preferred.

Combinatorial cloning is disclosed generally in PCT Publication No. WO90/14430. Simply stated, the goal of combinatorial cloning is to transfer to a population of bacterial cells the immunological genetic capacity of a human cell, tissue or organ. It is preferred to employ cells, tissues or organs which are immunocompetent. Particularly useful sources include, without limitation, spleen, thymus, lymph nodes, bone marrow, tonsil and peripheral blood lymphocytes. The cells may be optionally RSV stimulated *in vitro*, or selected from donors which are known to have produced an immune response or donors who are HIV<sup>+</sup> but asymptomatic.

The genetic information isolated from the donor cells can be in the form of DNA or RNA and is conveniently amplified by Polymerase Chain Reaction (PCR) or similar techniques. When isolated as RNA the genetic information is preferably converted into cDNA by reverse transcription prior to amplification. The amplification can be generalized or more specifically tailored. For example, by a careful selection of PCR primer sequences, selective amplification of immunoglobulin genes or subsets within that class of genes can be achieved.

Once the component gene sequences are obtained, in this case the genes encoding the variable regions of the various heavy and light antibody chains, the light and heavy chain genes are associated in random combinations to form a random combinatorial library. Various recombinant DNA vector systems have been described to facilitate combinatorial cloning [see: PCT Publication No. WO90/14430 supra; Scott and Smith, Science 249:386-406 (1990); or U. S. Patent 5,223,409]. Having generated the combinatorial library, the products can, after expression, be conveniently screened by biopanning with RSV F protein or, if necessary, by epitope blocked biopanning as described in more detail below.

As described herein, it is preferred to use single chain antibodies for combinatorial cloning and screening and then to convert them to full length mAbs after selection of the desired candidate molecules. However, Fab fragments of mAbs can also be used for cloning and screening.

### *III. Antibody Fragments.*

The present invention contemplates the use of scFv, Fab, or F(ab')<sub>2</sub> fragments to derived full-length mAbs directed against the F protein of RSV. Although these

fragments may be independently useful as protective and therapeutic agents *in vivo* against RSV-mediated conditions or *in vitro* as part of an RSV diagnostic, they are employed herein as a component of a reshaped human antibody. A scFv fragment contains the light and heavy chain variable regions joined by a linker of about 12 amino acids in either a light-linker-heavy or a heavy-linker-light orientation. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and a F(ab')<sub>2</sub> fragment is the fragment formed by two Fab fragments bound by additional disulfide bonds. RSV binding monoclonal antibodies provide sources of scFv or Fab fragments which can be obtained from a combinatorial phage library [see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994) or Barbas et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992), which are both hereby incorporated by reference in their entireties].

IV. *Anti-RSV Antibody Amino Acid and Nucleotide Sequences of Interest.*

The Fab G $\lambda$ -1 or other antibodies described herein may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention thus provides variable light chain and variable heavy chain sequences from the RSV human Fab G $\lambda$ -1A and sequences derived therefrom. The heavy chain variable region of Fab G $\lambda$ -1A is illustrated by Figs. 4, 8A-8F and 10A-10B [SEQ ID NOS: 3-4, 13 and 15].

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions can be used to create restriction enzyme sites which would facilitate insertion of mutagenized CDR (and/or framework) regions. These CDR-encoding regions may be used in the construction of reshaped human antibodies of this invention.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the human framework regions surrounding the CDR-encoding regions. Such sequences include all nucleic acid sequences which

by virtue of the redundancy of the genetic code are capable of encoding the same amino acid sequence as given in Figs. 3 and 4 [SEQ ID NOS: 2 and 4]. Figs. 6 and 7 [SEQ ID NOS: 5-12] provide representations of such sequences. Other useful DNA sequences encompassed by this invention include those sequences which hybridize under stringent hybridization conditions [See: T. Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences encoding the G $\lambda$ -1 antibodies (e.g., sequences of Figs. 3, 4, 8A-8F through 11 [SEQ ID NOS: 1-4, 13-16]) and which retain the antigen binding properties of those antibodies. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

*V. Altered Immunoglobulin Coding Regions and Altered Antibodies.*

Altered immunoglobulin coding regions encode altered antibodies which include engineered antibodies such as chimeric antibodies, humanized, reshaped, and immunologically edited human antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions in the form of scFv regions that encode peptides having the antigen specificity of an RSV antibody, preferably a high affinity antibody such as provided by the present invention, inserted into an acceptor immunoglobulin partner.

When the acceptor is an immunoglobulin partner, as defined above, it includes a sequence encoding a second

antibody region of interest, for example, an Fc region. Immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of RSV may be designed to elicit enhanced binding with the same antibody.

The immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. For example the reshaped human antibody having the signal sequence and CDRs derived from the Fab G $\lambda$ -1 heavy chain sequence, may have the original signal peptide replaced with another signal sequence such as the Campath leader sequence [Page, M. J. et al., BioTechnology 9:64-68(1991)].

An exemplary altered antibody, a reshaped human antibody, contains a variable heavy and the entire light chain peptide or protein sequence having the antigen specificity of Fab G $\lambda$ -1, fused to the constant heavy regions C<sub>H-1</sub>-C<sub>H-3</sub> derived from a second human antibody.

In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or C<sub>H-2</sub>C<sub>H-3</sub> domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')<sub>2</sub> fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F<sub>v</sub> or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor Fab G $\lambda$ -1. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

Whenever the immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-RSV antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the

acceptor mAb light and/or heavy variable domain  
framework region at the nucleic acid or amino acid  
levels, or the donor CDR regions may be made in order to  
retain donor antibody antigen binding specificity or to  
5 reduce potential immunogenicity.

Such engineered antibodies are designed to employ  
one (or both) of the variable heavy and/or light chains  
of the RSV mAb (optionally modified as described) or one  
or more of the below-identified heavy or light chain  
10 CDRs. The engineered antibodies of the invention are  
neutralizing, i.e., they desirably inhibit virus growth  
*in vitro* and *in vivo* in animal models of RSV infection.

Such engineered antibodies may include a reshaped  
human antibody containing the human heavy and light  
15 chain constant regions fused to the RSV antibody  
functional fragments. A suitable human (or other  
animal) acceptor antibody may be one selected from a  
conventional database, e.g., the KABAT<sup>®</sup> database, Los  
Alamos database, and Swiss Protein database, by homology  
20 to the nucleotide and amino acid sequences of the donor  
antibody. A human antibody characterized by a homology  
to the framework regions of the donor antibody (on an  
amino acid basis) may be suitable to provide a heavy  
chain constant region and/or a heavy chain variable  
25 framework region for insertion of the donor CDRs. A  
suitable acceptor antibody capable of donating light  
chain constant or variable framework regions may be  
selected in a similar manner. It should be noted that  
the acceptor antibody heavy and light chains are not  
30 required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant  
regions are selected from human immunoglobulin classes  
and isotypes, such as IgG (subtypes 1 through 4), IgM,  
IgA and IgE. The Fc domains are not limited to native

sequences, but include mutant variants known in the art that alter function. For example, mutations have been described in the Fc domains of certain IgG antibodies that reduce Fc-mediated complement and Fc receptor binding [see, e.g., A. R. Duncan *et al.*, Nature, 332:563-564 (1988); A. R. Duncan and G. Winter, Nature, 332:738-740 (1988); M.-L. Alegre *et al.*, J. Immunol., 148:3461-3468 (1992); M.-H. Tao *et al.*, J. Exp. Med., 178:661-667 (1993); and V. Xu *et al.* J. Biol. Chem., 269:3469-2374 (1994)]; alter clearance rate [J.-K. Kim *et al.*, Eur. J. Immunol., 24:542-548 (1994)]; and reduce structural heterogeneity [S. Angal *et al.*, Mol. Immunol. 30:105-108 (1993)]. Also, other modifications are possible such as oligomerization of the antibody by addition of the tailpiece segment of IgM and other mutations [R. I. F. Smith and S. L. Morrison, Biotechnology 12:683-688 (1994); R. I. F. Smith *et al.*, J. Immunol., 154: 2226-2236 (1995)] or addition of the tailpiece segment of IgA [I. Kariv *et al.*, J. Immunol., 157: 29-38 (1996)]. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

The altered antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of RSV mediated diseases in man, or for diagnostic uses.

It will be understood by those skilled in the art that an altered antibody may be further modified by changes in variable domain amino acids without

necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both. Particularly preferred is the immunological editing of such reconstructed sequences as illustrated in the examples herein.

In addition, the variable or constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention, as described above. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement [see, e.g., Angal et al., Mol. Immunol, 30:105-108 (1993); Xu et al., J. Biol. Chem, 269:3469-3474 (1994); and Winter et al., EP 307,434-B].

Such antibodies are useful in the prevention and treatment of RSV mediated disorders, as discussed below.

#### VI. *Production of Altered antibodies and Engineered Antibodies.*

The resulting reshaped human antibodies of this invention can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is

identical to the first except insofar as the coding sequences and selectable markers are concerned. This ensures as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and  
5 light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to  
10 create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The production of the  
15 antibody which includes the association of both the recombinant heavy chain and light chain is measured in the culture by an appropriate assay, such as an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Similar conventional techniques may be employed  
20 to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of  
25 skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Any vector,  
30 which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated, may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vectors. Preferred vectors include for example plasmids pCD or pCN. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a polyadenylation (polyA) signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., Molecular Cloning (A Laboratory Manual), 2nd edit., Cold Spring Harbor Laboratory (1989).

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant scFvs, Fabs and MAbs of the present invention [see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)]. The tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form does not pose as great a concern because Fabs are not normally glycosylated and can be engineered for exported expression, thereby reducing the high concentration that facilitates misfolding.

Nevertheless, any recombinant Fab produced in a bacterial cell would be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced and exported in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems [see, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein].

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of reshaped antibodies may utilize expression in a transgenic animal. An exemplary systems is described in U. S. Patent No. 4,873,316. The expression system described in that reference uses the animal's casein promoter and,

when transgenically incorporated into a mammal, permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. At present, conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the altered antibody to RSV. Additionally, other *in vitro* assays and *in vivo* animal models may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the altered antibody in the body despite the usual clearance mechanisms.

VII. *Therapeutic/Prophylactic Uses.*

This invention also relates to a method of treating humans experiencing RSV-related symptoms which comprises administering an effective dose of antibodies including one or more of the antibodies (altered, reshaped, monoclonal, etc.) described herein or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by binding to RSV and thus subsequently blocking RSV propagation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing RSV infection. For example, longer treatments may be desirable when treating seasonal episodes or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The altered antibodies, antibodies and fragments thereof of this invention may also be used alone or in conjunction with other antibodies, particularly human or humanized mAbs reactive with other epitopes on the F  
5 protein or other RSV target antigens as prophylactic agents.

The mode of administration of the therapeutic and prophylactic agents of the invention may be any suitable route which delivers the agent to the host. The altered  
10 antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

15 Therapeutic and prophylactic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the altered antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. An aqueous  
20 suspension or solution containing the antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or  
25 a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These  
30 solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and

buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15  
5 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain  
10 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 80 mg, or more preferably, about 5 mg to about 75 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion  
15 could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 75 and preferably 5 to about 50 mg/ml of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known  
20 or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic and  
25 prophylactic agents of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human  
30 or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably i.v. or i.m. (intramuscularly).

Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of RSV mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISAs and other conventional assay formats for the measurement of RSV levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis et al., cited above, or Sambrook et al., cited above.

**Example 1:** Isolation of G $\lambda$ -1 scFv-1

Single chain (sc) Fv libraries were prepared from an individual purposely exposed to RSV and selected against recombinant RSV F-protein following described procedures [R. H. Jackson *et al*, in *Protein Engineering, A Practical Approach*, A. R. Rees *et al* eds, Oxford University Press, chapter 12, pp. 277-301, 1992; H. R. Hoogenboom *et al.*, Nucl. Acid Res., 19: 4133-4137 (1991); J. D. Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991)]. Briefly, lymphocytes were isolated from a blood sample taken 15 days post exposure. RNA isolated from the lymphocytes was used for preparation of scFv encoding repertoires for phage display. Sets of V-region primers were paired with constant region primers for heavy chain domain 1 IgG and IgM and light chain C- $\kappa$  and C- $\lambda$  and then linked in a scFv VH-VL orientation with a 15 amino acid spacer (glycine<sub>4</sub>-serine)<sub>3</sub> [SEQ ID NO: 21] by overlap PCR [see J. D. Marks *et al.*, cited above, for description of the primers].

The resulting four scFv repertoires (V- $\kappa$  with IgG and IgM, V- $\lambda$  with IgG and IgM) were cloned into a phagemid vector similar to pHEN1 [H. R. Hoogenboom *et al.*, cited above] resulting in fusion of the scFvs to gene III of phage fd. The vector was then transformed into *E. coli* (e.g., strain TG1) by electroporation to yield the corresponding phagemid libraries.

Phage libraries displaying the scFv-gene 3 fusions were prepared by infection of each of the plasmid libraries with the M13K07 helper phage [R. H. Jackson, cited above] and were individually subjected to 2 rounds of panning against recombinant F-protein coated onto plastic. In the first round, 10<sup>11</sup> phage in 2.5 ml phosphate buffered saline (PBS)/2% Marval™ non-fat dry

milk were incubated for 90 minutes in a tube coated with 5 µg/ml of F-protein [described in P. Tsui *et al*, J. Immunol., 157:772-780 (1996)] followed by 1 wash with 10x PBS/0.05% Tween 20 and a second wash with 10x PBS alone. Bound phage were eluted with 10 mM triethylamine and the eluate was neutralized with 1 M Tris-HCl, pH 7.4. The eluted phage were amplified and subjected to a similar second round of panning, except that the concentration of F-protein for coating was 2 µg/ml and the wash buffer contained 20x PBS.

*E. coli* were infected with the eluted phage and 96 colonies from each starting library were superinfected with helper phage and screened for F-protein binding activity. Only four positive clones were obtained from the 2 IgM libraries, whereas 41 positives were observed for the IgG libraries. By partial sequence analysis, all of the clones carried one of three different heavy chains. Complete sequences were obtained for the heavy and light chain V-regions for six clones, all from the IgG libraries.

Serial dilutions of titered phage stocks of each of these six clones were tested by ELISA for binding to recombinant F-protein and to RSV infected cell lysate. All showed binding to F-protein with the phage designated Gλ-1 showing the best activity. However, Gλ-1 and three other clones showed little binding to the RSV lysate.

Three clones: Gλ-1, Gλ-3 (lysate binding positive), and Gκ-1 (lysate binding negative), where "κ" and "λ" designate the class of the light chain, were characterized further for competition of their binding by F-protein specific neutralizing monoclonal antibodies, and their ability to inhibit virus

infection. The neutralizing mAbs RSV19 and B4 described in International patent publication No. WO92/04381, published March 19, 1992, and International patent publication No. WO93/20210, published October 14, 1993, recognize distinct epitopes on the F-protein. GK-1 was strongly inhibited by both antibodies. G $\lambda$ -1 was significantly inhibited by B4 only. GK-3 was not inhibited by either antibody (shown for G $\lambda$ -1 only; see Figs. 1A and 1B). In initial assays (Table I, experiments 1-3), all three clones showed neutralizing activity *in vitro*, with G $\lambda$ -1 being the most potent (Fig. 2, a graph of experiment 2), while control wild-type phage (M13K07) not displaying scFv had no effect.

To address the possibility that neutralization might result just from phage coating of virus, irrespective of epitope, a phage preparation of the non-neutralizing Fab 5-16 was tested in the same assay. In three out of four assays, this preparation also showed good neutralization activity, as did the control phage in two of these assays (Table I, experiments 4-7). This confounding observation of variable neutralization by both Fab 5-16 and control M13K07 phage rendered the viral neutralization studies inconclusive.

Table I

Phage Sample	Virus Neutralization ( $IC_{50} \times 10^{-1}$ ) <sup>1</sup> (aru or kru/ml) <sup>2</sup>						
	Experiment #						
	1	2	3	4	5	6	7
GK-1 a	1,600		<300				
b				<10	<7		
Gλ-1 a		80	<300				
b				8.1	11		
c							120
Gλ-3 a		900	<300	180			
b					<7	10	
c							730
M13K07a			>10 <sup>5</sup>	>10 <sup>5</sup>		>5,000	
b					+all dil.	+all dil.	>10 <sup>5</sup>
Fab 5-19a				>10 <sup>5</sup>	40	180	
b							3.5

## Legend:

<sup>1</sup> Assay according to M. J. Cannon, J. Virol. Meth., 16:293-301. Virus at 100 infectious centers/well was incubated with dilutions of the indicated phage for 1 hr and then added to susceptible cells for 3 hr. The virus/phage solution was aspirated and replaced with fresh medium and the cells were incubated overnight before peroxidase staining for virus infected cells.

<sup>2</sup> aru = ampicillin resistance units, a measure of phagmid containing particles.

kru = kanamycin resistance units, a measure of particles containing the phage genome (for the M13K07 control only).

In the face of these results, made more ambiguous by the dependence of all assays on phage stocks verses antibody proteins of known concentration, G $\lambda$ -1 was selected as the most likely candidate for a potent neutralizing antibody based on (1) its apparent better binding to F-protein, (2) its selective inhibition of binding by the B4 antibody, and (3) its suggested activity over background in the virus neutralization assay.

10

**Example 2:** Conversion of G $\lambda$ -1 scFV to mAb Version A

The DNA and encoded protein sequences of the VH and VL regions of G $\lambda$ -1 are shown in Figs. 3 [SEQ ID NOS: 1 and 2] and 4 [SEQ ID NOS: 3 and 4], respectively. For expression in mammalian cells, the heavy chain variable region and the light chain variable region from the G $\lambda$ -1 plasmid were cloned into derivatives of plasmid pCDN [Nambi, A. et al., Mol. Cell. Biochem., 131:75-86 (1994)] in which the expression of the antibody chain is driven by the cytomegalovirus promoter (CMV) promoter. Plasmid pCD-HC68B is used for expressing full length heavy chains and plasmid pCN-HuLC, for expressing full length light chains.

In the initial constructs, changes in the sequence at the amino terminus were introduced by the PCR primers used for cloning the light chain and heavy chain variable regions from plasmid G $\lambda$ -1. In these constructs, the peptide signal sequence for both the heavy and light chains is derived from the Campath light chain [M. J. Page et al., Biotechnology 9: 64-68 (1991)]. The heavy chain of G $\lambda$ -1 was PCR amplified from G $\lambda$ -1 phagemid DNA, using primers for the amino terminus and framework 4 of the variable region. The resulting

PCR fragment was cut with XhoI (site introduced by the amino terminus primer) and BstEII (naturally occurring site in framework 4), and cloned into an intermediate vector, F4HCV, at the XhoI/BstEII sites.

5           This cloning grafted the variable region of G $\lambda$ -1 onto the constant region of another anti-RSV heavy chain 194-F4 [cloned at SmithKline Beecham from a human hybridoma]. This intermediate clone was cut with XhoI and Bsp120I, and introduced into the same sites in pCD-  
10 HC68B. The XhoI site is introduced at the amino terminus by the PCR primer and, when cloned into pCD-HC68B at the same site is preceded in frame by the Campath leader sequence. The Bsp120I site is a  
15 naturally occurring, highly conserved sequence at the beginning of the C<sub>H-1</sub> domain, and when cloned into pCD-HC68B at the same site, is in frame with the remaining sequence for the C<sub>H-1</sub> through C<sub>H-3</sub> regions of human IgG<sub>1</sub>. In the resulting construct, G $\lambda$ -1Apcd (Figs. 8A-8F [SEQ ID NO: 13]), the amino acids immediately following the  
20 Campath leader are EVQLLE [SEQ ID NO: 17], where the residues LE are encoded by the nucleotide sequence for the XhoI cloning site.

          The light chain of G $\lambda$ -1 was PCR amplified from the G $\lambda$ -1 phagemid DNA, using primers for the amino terminus  
25 and framework 4 of the variable region. The resulting PCR fragment was cut with SacI (site introduced by the amino terminus primer) and AvrII (naturally occurring site in framework 4), and cloned into 43-lpcn at the SacI/AvrII sites. This cloning grafted the variable  
30 region of G $\lambda$ -1, in frame, onto the constant region of another anti-RSV lambda light chain 43 [P. Tsui et al., J. Immunol., 157: 772-780 (1996)], which had been cloned at SmithKline Beecham from a combinatorial library derived from RNA isolated from human spleen. The SacI

site is introduced at the amino terminus by the PCR primer and, when cloned into 43pcn at the same site, is preceded in frame by the Campath leader sequence. The first two amino acids of the mature light chain are therefore deleted. In the resulting construct, G $\lambda$ -1Apcn (Figs. 9A-9E [SEQ ID NO: 14]), the first two amino acids immediately following the leader are EL, where the residues EL are encoded by the nucleotide sequence for the SacI cloning site.

The nucleotide sequences of the plasmids G $\lambda$ -1Apcd and G $\lambda$ -1Apcn are shown in Figs. 8A-8F [SEQ ID NO: 13] and 9A-9E [SEQ ID NO: 14] respectively. This set of vectors was used to produce antibody G $\lambda$ -1A in COS cells and in CHO cells.

### **Example 3: Cloning Of The Corrected G $\lambda$ -1 Heavy and Light Chains**

In cloning the variable region of the G $\lambda$ -1 heavy chain from the single chain Fv (scFv) format into the full length format, the fifth amino acid at the amino terminus was changed from Val to Leu, for cloning purposes. To correct this change, PCR primers were designed for the amino terminus of the G $\lambda$ -1 heavy chain cloned into pCD, which reverted the fifth amino acid back to Val. The correction was introduced via the PCR overlap technique using the correction primers and primers annealing to sequences within the CMV promoter and the C<sub>H-2</sub> constant region as the outside 5' and 3' primers, respectfully. The final PCR product was digested with restriction enzymes, EcoRI and Bsp120I, and cloned into the G $\lambda$ -1Apcd vector at the same sites to create G $\lambda$ -1Bpcd.

The final construct was sequenced to verify that the amino terminus of the heavy chain had been corrected from EVQLLE [SEQ ID NO: 17] to EVQLVE [SEQ ID NO: 18] (see Fig 6). The nucleotide sequence of coding region  
5 for the corrected heavy chain, G $\lambda$ -1B, is shown in Figs. 10A-10B [SEQ ID NO: 15].

In cloning the variable region of the G $\lambda$ -1 light chain from the scFv format into the full length format, changes were introduced at the amino terminus for  
10 cloning purposes. Specifically, the first 2 amino acids (Gln and Ser) of the light chain were deleted and the third amino acid was changed from Val to Glu. To correct these changes, PCR primers were designed for the amino terminus of the G $\lambda$ -1 light chain cloned into pCN,  
15 which replaced the two deleted amino acids (Gln and Ser) and reverted the third amino acid back to Val. The corrections were introduced via the PCR overlap technique using the correction primers and primers annealing to sequences within the CMV promoter and the  $\lambda$   
20 constant region as the outside 5' and 3' primers, respectfully. The final PCR product was digested with restriction enzymes, EcoRI and AvrII and cloned into the G $\lambda$ -1Apcn vector at the same sites to create G $\lambda$ -1Bpcn.

The final construct was sequenced to verify that  
25 the amino terminus of the light chain had been corrected from --EL to QSVL (amino acids 1-4 of SEQ ID NO: 10).

The nucleotide sequence of coding region for the corrected light chain, G $\lambda$ -1B, is shown in Fig. 11 [SEQ ID NO: 16]. This vector G $\lambda$ -1Bpcn, was used with G $\lambda$ -  
30 1Bpcd to produce antibody G $\lambda$ -1B, in COS cells and in CHO cells.

**Example 4:** Production of G $\lambda$ -1 mABs in Mammalian Cells

For initial characterization, the mAb constructs for each version, G $\lambda$ -1A heavy and light chain, G $\lambda$ -1B heavy and light chain, were expressed in COS cells essentially as described in Current Protocols in Molecular Biology, eds F. M. Ausubel et al., 1988, John Wiley & Sons, vol. 1, section 9.1. On day 1 after the transfection, the culture growth medium was replaced with a serum-free medium [SmithKline Beecham] which was changed on day 3. Similar satisfactory results are obtained using a publicly available medium, DMEM supplemented with ITS<sup>TM</sup> Premix, an insulin, transferrin, selenium mixture (Collaborative Research, Bedford, MA) and 1 mg/ml bovine serum albumin (BSA).

The mAb was prepared from the day 3 + day 5 conditioned medium by standard protein A affinity chromatography methods (e.g., as described in Protocols in Molecular Biology) using, for example, Prosep A affinity resin (Bioprocessing Ltd., UK).

To produce larger quantities of the G $\lambda$ -1B mAB (100-200 mgs), the vectors were introduced into a proprietary CHO cell system. However, similar results will be obtained using dhfr<sup>-</sup> CHO cells as previously described [P. Hensley et al., J. Biol. Chem., 269:23949-23958 (1994)]. Briefly, a total of 30  $\mu$ g of linearized plasmid DNA (15  $\mu$ g each of the A or B set of heavy chain and light chain vectors) is electroporated into  $1 \times 10^7$  cells. The cells are initially selected in nucleoside-free medium in 96 well plates. After three to four weeks, media from growth positive wells is screened for human immunoglobulin using an ELISA assay. The highest expressing colonies are expanded and selected in increasing concentrations of methotrexate for

amplification of the transfected vectors. The antibody is purified from conditioned medium by standard procedures using protein A affinity chromatography (Protein A sepharose, Pharmacia) followed by size  
5 exclusion chromatography (Superdex 200, Pharmacia).

The concentration and the antigen binding activity of the eluted antibody are measured by ELISA. The antibody containing fractions are pooled and further purified by size exclusion chromatography. As expected  
10 for any such antibody, by SDS-PAGE, the predominant protein product migrated at approximately 150 kd under non-reducing conditions and as two bands of 50 and 25 kd under reducing conditions. For antibody produced in CHO cells, the purity was > 90%, as judged by SDS-PAGE, and  
15 the concentration was accurately determined by amino acid analysis.

**Example 5:** Binding of the G $\lambda$ -1 mABs to recombinant F protein

20 Binding of the G $\lambda$ -1 mABs to recombinant F protein was measured in a standard solid phase ELISA. Antigen diluted in PBS pH 7.0 was adsorbed onto polystyrene round-bottom microplates (Dynatech, Immunolon II) for 18 hours. Wells were then aspirated and blocked with 0.5%  
25 boiled casein (BC) in PBS containing 1% Tween 20 (PBS/0.05% BC) for two hours. Antibodies (50  $\mu$ l/well) were diluted to varying concentrations in PBS/0.5% BC containing 0.025% Tween 20 and incubated in antigen coated wells for one hour. Plates were washed three  
30 times with PBS containing 0.05% Tween 20, using a Titertek 320 microplate washer, followed by addition of HRP-labelled protein A/G (50  $\mu$ l) diluted 1:5000. After washing three times, TMBBlue substrate (TSI, #TM102) was added and plates were incubated an additional 15

minutes. The reaction was stopped by addition of 1 N H<sub>2</sub>SO<sub>4</sub> and absorbance read at 450 nm using a Biotek ELISA reader.

The antigen binding epitope of the G $\lambda$ -1 mABs was examined in a competition ELISA. The G $\lambda$ -1 mABs were mixed with increasing concentrations of RSMU19 or B4, two potent neutralizing mAbs [Tempest et al., Biotech., 9: 266-271 (1991); Kennedy et al., J. Gen. Virol., 69: 3023-3032 (1988)] and added to F protein-coated wells. The epitope regions recognized by mAbs RSMU19 and B4 are quite distinct from each other as previously described in Arbiza et al., J. Gen. Virol., 73: 2225-2234 (1992). The concentration of the G $\lambda$ -1 mABs used in competition studies was determined previously to give 90% maximal binding to F antigen. Binding of the G $\lambda$ -1 mABs in the presence of other mABs was detected using HRP-labelled goat anti-human IgG. The reaction was developed as stated above.

The G $\lambda$ -1 mABs demonstrated potent binding to recombinant F (rF) protein by ELISA (EC<sub>50</sub> for mAB B = 2.6 ng/ml). Binding of the G $\lambda$ -1 mABs to rF protein was inhibited by mAb B4, for which the F protein amino acids critical for antigen recognition are amino acids 268, 272 and 275 of SEQ ID NO: 20). Binding of the G $\lambda$ -1 mABs to rF protein was not inhibited by mAb RSMU19, for which F protein amino acid 429 of SEQ ID NO: 20 is critical for antigen recognition. These results indicate that residues in the region of amino acids 255-275 of the F protein [SEQ ID NO: 20] are critical for G $\lambda$ -1 mAB recognition.

**Example 6:** In vitro Fusion-Inhibition Activity of the G $\lambda$ -1 mABs

The ability of the G $\lambda$ -1 mABs to inhibit virus-induced cell fusion was determined using a modification of the *in vitro* microneutralization assay [Beeler et al., J. Virol., 63:2941-2950 (1989)]. In this assay, 50  $\mu$ l of RS Long strain virus (10-100 TCID<sub>50</sub>/well [American Type Culture Collection ATCC VR-26] were mixed with 0.1 ml VERO cells (5X10<sup>3</sup>/well) [ATCC CCL-81] in Minimum Essential Media (MEM) containing 2% fetal calf serum (FCS), for 4 hours at 37°C, 5% CO<sub>2</sub>. Serial two-fold dilutions (in quadruplicate) of mAB (50  $\mu$ l) were then added to wells containing virus-infected cells. Control cultures contained cells incubated with virus only (positive virus control) or cells incubated with media alone.

Cultures were incubated at 37°C in 5% CO<sub>2</sub> for 6 days at which time cytopathic effects (CPE) in virus control wells were > 90%. Microscopic examination for cytopathic effects were confirmed by ELISA. Media was aspirated from cultures and replaced with 50  $\mu$ l of 90% methanol containing 0.6% H<sub>2</sub>O<sub>2</sub>. After 10 minutes, fixative was aspirated and plates were air dried overnight. Viral antigen was detected in the fixed cultures using 1  $\mu$ g/ml biotinylated RSCHB4 (a human Fc derivative of the bovine B4 mAb [SmithKline Beecham]), followed by HRP-labelled streptavidin (Boehringer-Mannheim) diluted 1:10,000. The reaction was developed using TMBblue and stopped by addition of 1N H<sub>2</sub>SO<sub>4</sub>. Absorbance was measured at 450 nm (O.D.<sub>450</sub>).

Fusion-inhibition titers were defined as the concentration of antibody which caused a 50% reduction in ELISA signal (ED<sub>50</sub>) as compared to virus controls.

Based on the curve generated in the ELISA by the standard virus titration, a 50% reduction in O.D.<sub>450</sub> corresponded to  $\geq$  90% reduction in virus titer. Calculation of the 50% point was based on regression analysis of the dose titration.

The G $\lambda$ -1 mABs demonstrated potent *in vitro* fusion-inhibition activity against type A RS Long strain virus (ED<sub>50</sub> for mAB B of 0.51  $\pm$  0.38  $\mu$ g/ml). In this *in vitro* fusion-inhibition assay, G $\lambda$ -1 mAB B was more active than the humanized mAB RSHZ19 (ED<sub>50</sub> of 0.4-3.0  $\mu$ g/ml) [Wyde et al., Pediatr. Res., 38(4):543-550] in comparative assays.

**Example 7:** In vivo Activity of G $\lambda$ -1 mAB B: Prophylaxis and Therapy in Balb/c Mouse Model

Balb/c mice (5/group) were inoculated intraperitoneally with doses ranging from 0.06 mg/kg to 5 mg/kg of G $\lambda$ -1 mAB B either 24 hours prior (prophylaxis) or 4 days after (therapy) intranasal infection with 10<sup>5</sup> PFU of the A2 strain of human RSV. Mice were sacrificed 5 days after infection. Lungs were harvested and homogenized to determine virus titers.

Virus was undetectable in the lungs of mice treated prophylactically with  $\geq$  1.25 mg/kg G $\lambda$ -1 mAB B either prophylactically or therapeutically. See Table II below. Significant viral clearance (2-3 log<sub>10</sub>) was also achieved in animals receiving 0.31 mg/kg G $\lambda$ -1 mAB B either prophylactically or therapeutically.

**Table II: G $\lambda$ -1 mAB B Prophylaxis and Therapy in Balb/c Mice**

	<u>Treatment</u>	<u>Dose</u> (mg/kg)	<u>Lung Virus Titer (log<sub>10</sub>/g lung)</u>	
			<u>Prophylaxis</u>	<u>Therapy</u>
5	<b>G<math>\lambda</math>-1 mAB B</b>	5	<1.7	<1.7
		1.25	<1.7	<1.7
		0.31	1.8 $\pm$ 0.3	2.9 $\pm$ 0.4
		0.06	4.3 $\pm$ 0.7	4.5 $\pm$ 0.3
10	<b>PBS</b>	-	4.8 $\pm$ 0.7	4.7 $\pm$ 0.2

The G $\lambda$ -1 mABs have potent antiviral activity *in vitro* against a broad range of native RSV isolates of both type A and B, and show prophylactic and therapeutic efficacy *in vivo* in animal models. Thus, the G $\lambda$ -1 mABs are candidates for therapeutic, prophylactic, and diagnostic application in man.

Numerous modifications and variations of the present invention may be made by one of skill in the art in view of the invention described herein. Such modifications are believed to be encompassed by the specification and claims of the present invention. All references cited above are incorporated by reference herein.

## WHAT IS CLAIMED IS:

1. A human monoclonal antibody and functional fragments thereof, specifically reactive with an F protein epitope of Respiratory Syncytial Virus and capable of neutralizing infection by said virus selected from the group consisting of G $\lambda$ -1A and G $\lambda$ -1B.

2. The monoclonal antibody according to Claim 1 which comprises the light chain amino acid sequence of Fig. 3 SEQ ID NO: 2 and the heavy chain amino acid sequence of Fig. 4 SEQ ID NO: 4.

3. The monoclonal antibody according to Claim 1 which comprises the light chain amino acid sequence encoded by the DNA sequence of Fig. 11 SEQ ID NO: 16 and the heavy chain amino acid sequence encoded by the DNA sequence of Figs. 10A-10B SEQ ID NO: 15.

4. The monoclonal antibody according to Claim 1 wherein said fragment is selected from the group consisting of Fv, Fab and F(ab')<sub>2</sub>.

5. An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid sequence encoding any of the human monoclonal antibodies, altered antibodies and CDRs of any of the claims 1-4;

(b) a nucleic acid complementary to any of the sequences in (a); and

(c) a nucleic acid sequence of 18 or more nucleotides capable of hybridizing to the CDRs of any of claims 1-4 under stringent conditions.

6. The isolated nucleic acid molecule according to Claim 5 comprising the sequences of Figs. 8A-8F and 9A-9E SEQ ID NOS: 13 and 14, or Figs. 10A-10B and 11 SEQ ID NOS: 15 and 16.

7. A recombinant plasmid comprising the nucleic acid sequences of any of Claims 5 or 6.

8. A host cell comprising the plasmid of Claim 7.

9. A process for the production of a human antibody specific for RSV comprising culturing the host cell of Claim 8 in a medium under suitable conditions of time temperature and pH and recovering the antibody so produced.

10. A method of detecting RSV comprising contacting a source suspected of containing RSV with a diagnostically effective amount of the monoclonal antibody of Claim 1 and determining whether the monoclonal antibody binds to the source.

11. A method for providing passive immunotherapy to RSV disease in a human, comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of Claim 1.

12. The method according to Claim 11 wherein the passive immunotherapy is provided prophylactically.

13. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective

amount of the monoclonal antibody of Claim 1 in a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the monoclonal antibody of Claim 1 in combination with at least one additional monoclonal antibody.

15. The pharmaceutical composition according to Claim 14 wherein said additional monoclonal antibody is an anti-RSV antibody distinguished from the antibody of Claim 1 by virtue of being reactive with a different epitope of the RSV F protein antigen.

Fig. 1A

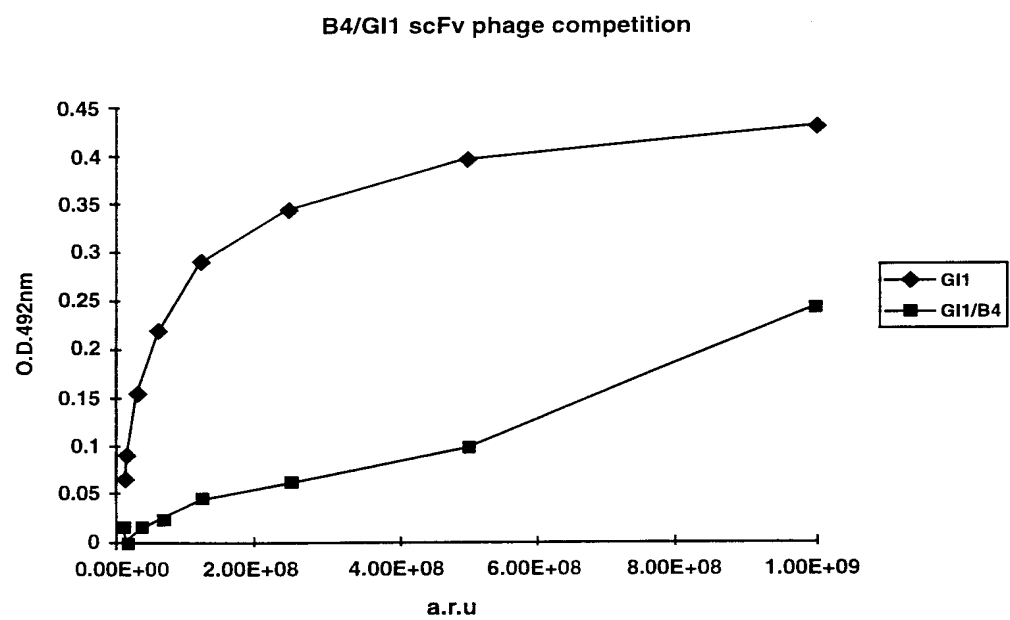
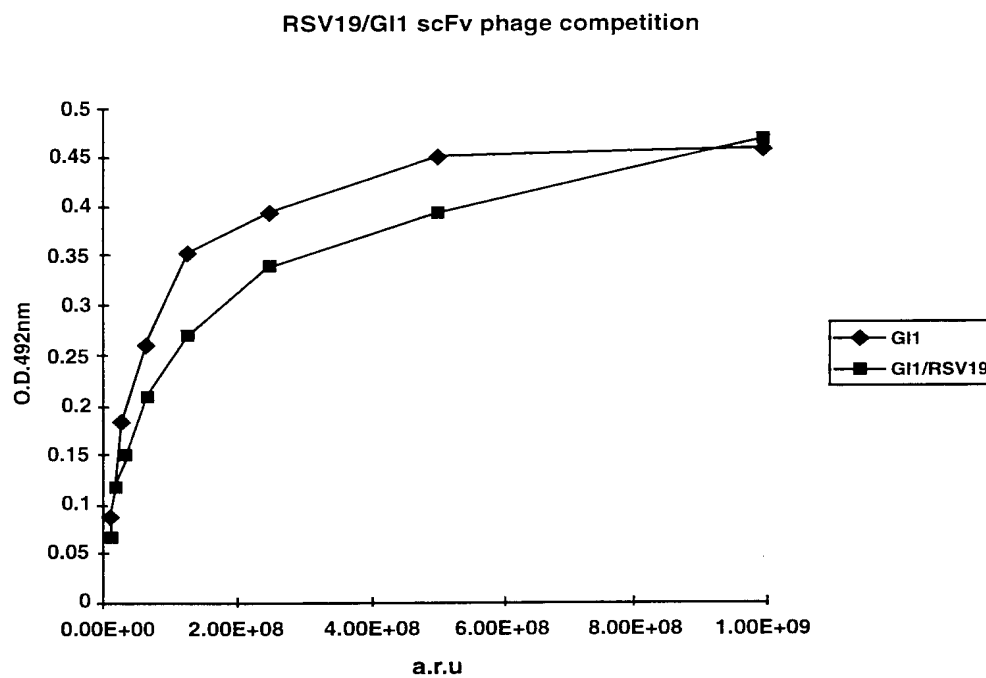


Fig. 1B

Fig. 2

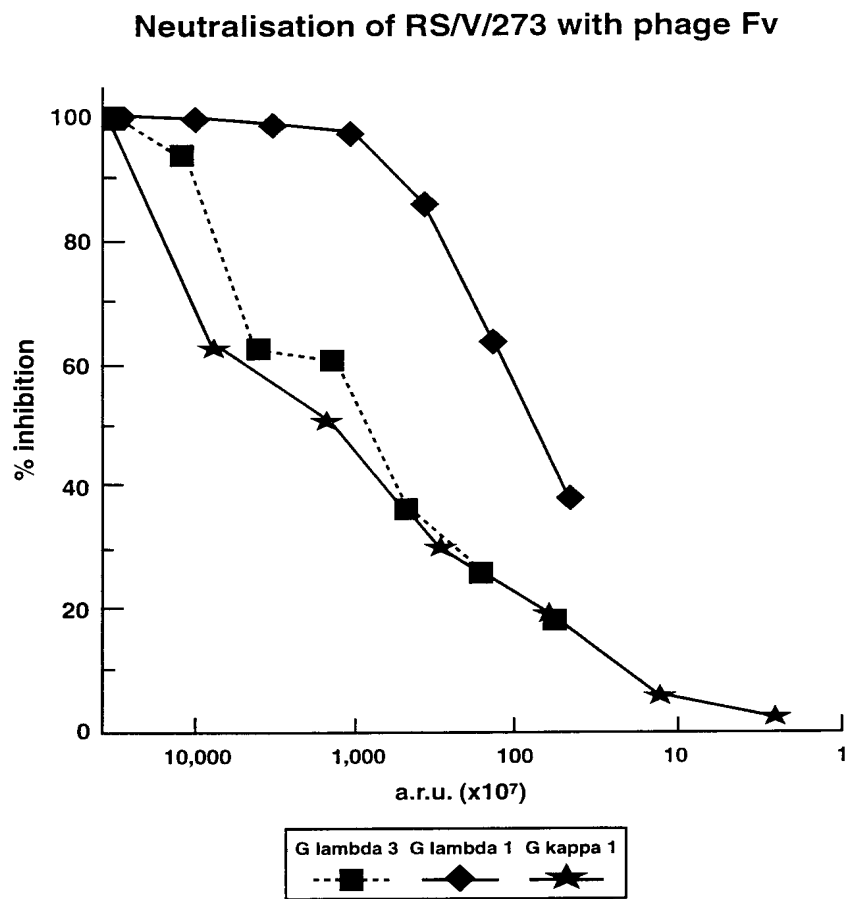


FIGURE 3

1	CAGTCTGTGTTGACGCAGCCGCCCTCAGTCTCTGCGGCCCCAGGACAGAA	50
	Q S V L T Q P P S V S A A P G Q K	
51	GGTCACCATCTCCTGCACTGGGAGCAGCTCCAACCTCGGGGCAGGTTATG	100
	V T I S C T G S S S N L G A G Y D	
101	ATGTTCACTGGTACCGGCAACTTCCAGGGACAGCCCCCAAACCTCCTCATC	150
	V H W Y R Q L P G T A P K L L I	
151	TATGATAACAACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTC	200
	Y D N N N R P S G V P D R F S G S	
201	CAAGTCTGGCCCCCTCAGCCTCCCTGGCCATCTCTGGGCTCCAGGCTGAGG	250
	K S G P S A S L A I S G L Q A E D	
251	ATGAGGCTGATTATTACTGCCAGTCCTATGACAGCAGCCTGAATGGTTAT	300
	E A D Y Y C Q S Y D S S L N G Y	
301	GTCTTCGGAACCTGGGACCCAGCTCACCGTCCTAGGT	336
	V F G T G T Q L T V L G	

FIGURE 4

1	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTC	50
	E V Q L V E S G G G L V Q P G G S	
51	CCTGAGACTCTCCTGCGCAGCCTCTGGAGTCTCCCTCAGTGGATAACAAGA	100
	L R L S C A A S G V S L S G Y K M	
101	TGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGTCTCTTCC	150
	N W V R Q A P G K G L E W V S S	
151	ATTACTGGTATGAGTAATTACATACACTACTCAGACTCAGTGAAGGGCCG	200
	I T G M S N Y I H Y S D S V K G R	
201	ATTCACCATCTCCAGAGACAACGCCATGAACTCACTGTATCTGCAAATGA	250
	F T I S R D N A M N S L Y L Q M N	
251	ACAGCCTGACAGCCGAGGACACGGGTGTTTATTATTGTGCGACACAACCG	300
	S L T A E D T G V Y Y C A T Q P	
301	GGGGAGCTGGCGCCTTTTGACCATTGGGGCCAGGGAACCCTGGTCACCGT	350
	G E L A P F D H W G Q G T L V T V	
351	CTCCTCA	357
	S S	

Figure 5

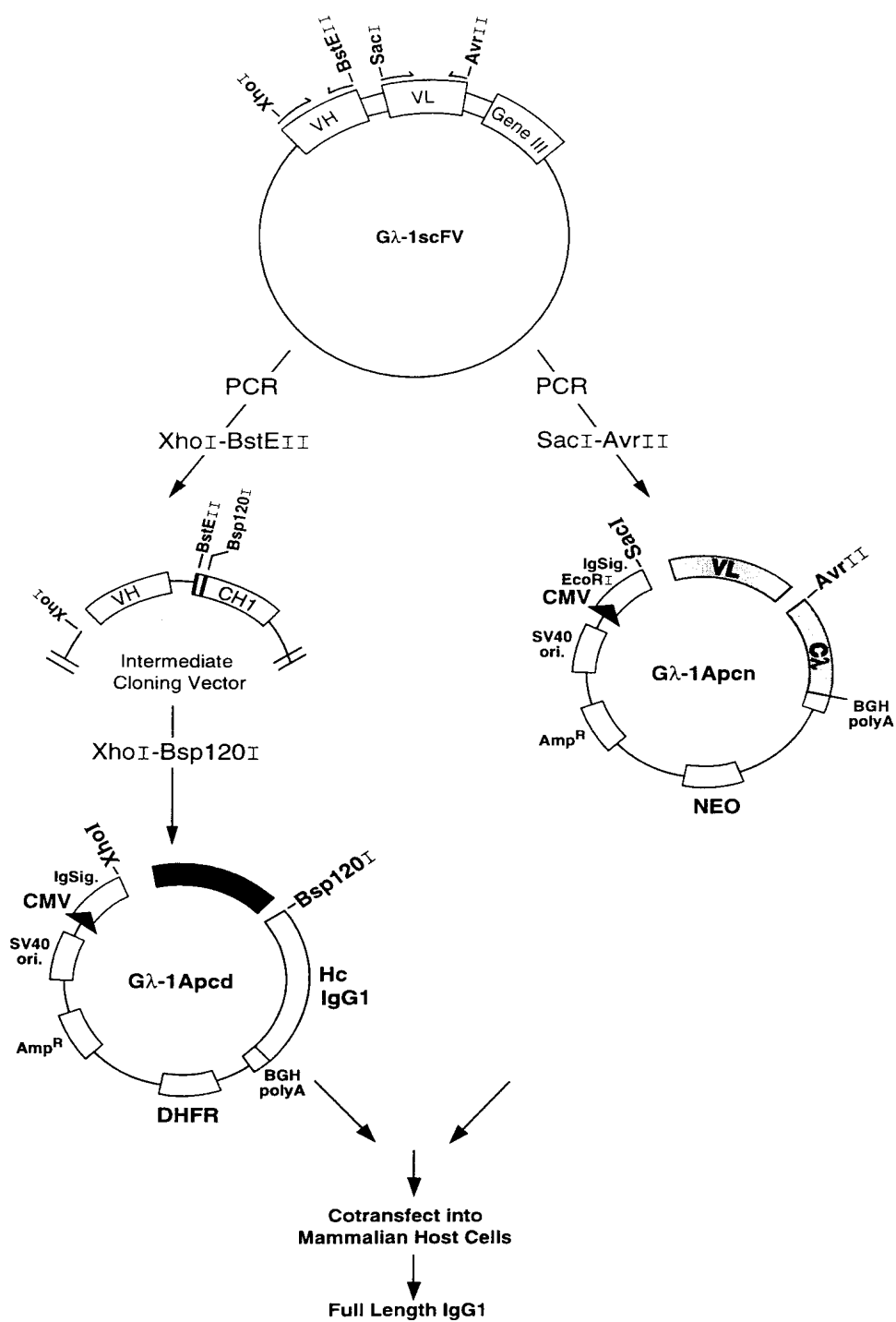


FIGURE 6

**Comparison of the Heavy Chain Amino Acid Sequences of the  
G $\lambda$ -1 single chain fv and mAbs**

Leader and Variable Regions

GL Dp58:	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
G $\lambda$ -1 scFv:	-----VSL-
G $\lambda$ -1A:	MGWSCIIILFLVATATGVHS---L-----
G $\lambda$ -1B:	-----V-----
	CDR1 CDR2
	-----
GL Dp58:	SYEMNWVRQAPGKGLEWVS <b>YISSSGSTIYYADSVKGR</b> FTISRDNAKNSLY
G $\lambda$ -1 scFv:	<b>G-K</b> -----S-TGMSNY-H-S-----M----
G $\lambda$ -1A:	-----
G $\lambda$ -1B:	-----
	CDR3
	-----
GL: Dp58:	LQMNSLRAEDTAVYYCAR
G $\lambda$ -1 scFv:	-----T---G----- <b>TQPGELAPFDHWG</b> QGTLVTVSS
G $\lambda$ -1A:	-----
G $\lambda$ -1B:	-----

## FIG 7

Comparison of the Light Chain Amino Acid Sequences of the G $\lambda$ -1A:  
single chain Fv and mAbs

## Leader and Variable Regions

		CDR1
		-----
GL DpL8:	QSVLTQPPSVSGAPGQRVTISCT <b>GGSSSNIG</b>	
G $\lambda$ -1 scFv:	-----A----K-----L-	
G $\lambda$ -1A:	<b>MGWSCIILFLVATATGVHS</b> E-----	
G $\lambda$ -1B:	-----QSV-----	
		CDR2
		-----
GL DpL8:	<b>AGYDVH</b> WYQQLPGTAPKLLIY <b>GNSNRPS</b> GVDPDRFSGSKSGTSASLAITGL	
G $\lambda$ -1scFv:	-----R-----D-N-----P-----S--	
G $\lambda$ -1A:	-----	
G $\lambda$ -1B:	-----	
		CDR3
		-----
GL DpL8:	QAEDEADYYC	
G $\lambda$ -1 scFv:	----- <b>QSYDSSLNGYV</b> FGTGTQLTVLG	
G $\lambda$ -1A:	-----	
G $\lambda$ -1B:	-----	

FIGURE 8A

1 gacgtcgcggccgctctagggcctccaaaaagcctcctcactacttctgg  
51 aatagctcagaggccgagggcgccctcggcctctgcataaataaaaaaaat  
101 tagtcagccatgcatggggcgagagaatgggcggaactgggcgaggttagg  
151 ggcgggatgggcgaggttagggcgggactatggttgctgactaattgag  
201 atgcatgctttgcataacttctgcctgctggggagcctggggactttccac  
251 acctggttgctgactaattgagatgcatgctttgcataacttctgcctgct  
301 ggggagcctggggactttccacaccctaactgacacacattccacagaat  
351 taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat  
401 caattacggggtcattagttcatagcccatatatggagttccgcgttaca  
451 taacttacggtaaatggcccgccctggctgaccgccaacgacccccgccc  
501 attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt  
551 tccattgacgtcaatgggtggactatttacggtaaaactgccacttggca  
601 gtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga  
651 cggtaaatggcccgccctggcattatgccagtacatgaccttatgggact  
701 ttctacttggcagtacatctacgtattagtcatcgctattaccatgggtg  
751 atgcggtttttggcagtacatcaatgggcgtggatagcggtttgactcacg  
801 gggatttccaagtctccaccccatgacgtcaatgggagtttgttttggc  
851 accaaaatcaacgggactttccaaaatgtcgtacaactccgccccattg  
901 acgcaaattgggcggtaggcgtgtacgggtgggaggtctatataagcagagc  
  
EcoRI  
951 tgggtacgtgaaccgtcagatcgccctggagacgccatcgaattctgagca  
1001 cacaggacctcaccatgggatggagctgtatcatcctcttcttggttagca  
M G W S C I I L F L V A  
Leader start  
  
XhoI  
1051 acagctacaggtgtccactccgaggtccaactgctcgagtctgggggagg  
T A T G V H S E V Q L L E S---  
Processed N-term

FIGURE 8B

1101 cttggtacagcctgggggggtccctgagactctcctgcgagcctctggag  
1151 tctccctcagtggatacaagatgaactgggtccgccagggtccaggggaag  
1201 gggctggaatgggtctcttccattactgggtatgagtaattacatacacta  
1251 ctcagactcagtgaagggccgattcaccatctccagagacaacgccatga  
1301 actcactgtatctgcaaataaacagcctgacagccgaggacacgggtgtt  
1351 tattattgtgcgacacaaccggggggagctggcgcccttttgaccattgggg  
  
                            BstEII  Bsp120I  
1401 ccagggaaccctgggtcaccgtctcctcagcctccaccaaggggcccatcgg  
      Q  G  T  L  V  T  V  S  S  /  
                            framework IV / CH1  
  
1451 tcttccccctggcacccctcctccaagagcacctctgggggacagcggcc  
1501 ctgggctgcctggtcaaggactacttccccgaaccggtgacgggtgtcgtg  
1551 gaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctac  
  
  BstEII  
1601 agtcctcaggactctactccctcagcagcgtgggtgaccgtgcctccagc  
1651 agcttgggacccagacctacatctgcaacgtgaatcacaagcccagcaa  
1701 caccaagggtggacaagaaagttgagcccaaattcttgtagacaaaactcaca  
1751 catgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttc  
1801 ctcttccccccaaaacccaaggacaccctcatgatctcccggaacctga  
1851 ggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagt  
1901 tcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccg  
1951 cgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcacgt  
2001 cctgcaccaggactggctgaatggcaaggagtacaagtgcaagggtctcca  
2051 acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggg  
2101 cagccccgagaaccacagggtgtacaccctgcccccatcccgggatgagct  
2151 gaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatccca

FIGURE 8C

2201 gcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactac  
2251 aagaccacgcctcccgtgctggactccgacggctccttcttcctctacag  
2301 caagctcacccgtggacaagagcaggtggcagcaggggaacgtcttctcat  
2351 gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc  
2401 tccctgtctccgggtaaatgatagatatctacgtatgatcagcctcgact  
S P G K \* C-term of heavy chain  
2451 gtgccttctagttgccagccatctgttggttgcccctcccccggtgccttc  
2501 cttgaccctggaaggtgccactcccactgtcctttcctaataaaaatgagg  
2551 aaattgcatcgcatgtgtctgagtaggtgtcattctattctgggggggtggg  
2601 gtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgc  
2651 tggggatgcggtgggctctatggaaccagctggggctcgacagcgctgga  
2701 tctcccgatccccagctttgcttctcaatttcttatttgcataatgagaa  
2751 aaaaaggaaaattaattttaacaccaattcagtagttgattgagcaaatg  
2801 cgttgccaaaaaggatgctttagagacagtggttctctgcacagataagga  
2851 caaacattattcagagggagtagccagagctgagactcctaagccagtga  
2901 gtggcacagcattctagggagaaatatgcttgctcatcacgaagcctgat  
2951 tccgtagagccacaccttggttaagggccaatctgctcacacaggatagag  
3001 agggcaggagccagggcagagcatataaggtgaggtaggatcagttgctc  
3051 ctcacatttgcttctgacatagttgtgttgaggagcttgatagcttggac  
3101 agctcagggtgcgatttcgcgcgcaaacttgacggcaatcctagcgtgaa  
3151 ggctggtaggattttatccccgctgccatcatgggttcgaccattgaactg  
3201 catcgtcgccgtgtcccaaaatatggggattggcaagaacggagacctac  
3251 cctggcctccgctcaggaacgagttcaagtacttccaaagaatgaccaca  
3301 acctcttcagtgggaaggtaaacagaatctgggtgattatgggtaggaaaac  
3351 ctggttctccattcctgagaagaatcgacctttaaggacagaattaata

FIGURE 8D

3401 tagttctcagtagagaaactcaaagaaccaccacgaggagctcatTTTTCTT  
3451 gccaaaagtttggatgatgccttaagacttattgaacaaccggaattggc  
3501 aagtaaagtagacatggtttggatagtcggaggcagttctgtttaccagg  
3551 aagccatgaatcaaccaggccaccttagactctttgtgacaaggatcatg  
3601 caggaatttgaaagtgacacgTTTTTCCCAGAAATTGATTTGGGGAAATA  
3651 taaacttctcccagaatacccaggcgctctctctgagggtccaggaggaaa  
3701 aaggcatcaagtataagtttgaagtctacgagaagaaagactaacaggaa  
3751 gatgctttcaagttctctgctccctcctaagctatgcatttttataag  
3801 accatgggactTTTTGCTGGCTTTAGATCAGCCTCGACTGTGCCTTCTAGT  
3851 tgccagccatctgttgTTTTGCCCTCCCCCGTGCCTTCCTTGACCCTGGA  
3901 aggtgccactcccactgtcTTTTCTAATAAAATGAGGAAATTGCATCGC  
3951 attgtctgagtaggtgtcattctattctgggggggtgggggtggggcaggac  
4001 agcaagggggaggattgggaagacaatagcaggcatgctggggatgcggt  
4051 gggctctatggaaccagctggggctcgatcgagtgtatgactgcggccgc  
4101 gatcccgctcgagagcttggcgtaatcatgggtcatagctgtttcctgtgtg  
4151 aaattgttatccgctcacaattccacacaacatacgagccggaagcataa  
4201 agtgtaaagcctgggggtgcctaatagtgagctaactcacattaattgcg  
4251 ttgcgctcactgcccgtttccagtcgggaaacctgtcgtgccagctgca  
4301 ttaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgct  
4351 cttccgcttcctcgctcactgactcgctgcgctcggtcggttcggctgcgg  
4401 cgagcgggtatcagctcactcaaaggcggtaatacggttatccacagaatc  
4451 aggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggcca  
4501 ggaaccgtaaaaaggccgcgttgctggcgTTTTTCCATAGGCTCCGCCCC  
4551 cctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaaccc  
4601 gacaggactataaagataccaggcgTTTTCCCCTGGAAGCTCCTCGTGC

FIGURE 8E

4651 gctctcctgttccgaccctgccgcttacccggatacctgtccgcctttctc  
4701 ccttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatctcag  
4751 ttcggtgtaggtcggttcgctccaagctgggctgtgtgcacgaaccccccg  
4801 ttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaac  
4851 ccggtaagacacgacttatcgccactggcagcagccactggtaacaggat  
4901 tagcagagcaggtatgtaggcggtgctacagagttcttgaagtgggtggc  
4951 ctaactacggctacactagaaggacagtatttggtatctgcgctctgctg  
5001 aagccagttaccttcggaaaaagagttggtagctcttgatccggcaaaca  
5051 aaccaccgctggtagcgggtgggtttttttgtttgcaagcagcagattacgc  
5101 gcagaaaaaaaaggatctcaagaagatcctttgatcttttctacgggggtct  
5151 gacgctcagtggaaacgaaaactcacgttaagggatttttggtcatgagatt  
5201 atcaaaaaggatcttcacctagatccttttaaattaaaaatgaagtttta  
5251 aatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgc  
5301 ttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccat  
5351 agttgcctgactccccgctcgtgtagataactacgatacgggaggggttac  
5401 catctggccccagtgctgcaatgataccgcgagaccacgctcacccggct  
5451 ccagatttatcagcaataaaccagccagccggaagggccgagcgcagaag  
5501 tggctctgcaactttatccgcctccatccagtctattaattggtgccggg  
5551 aagctagagtaagtagttcgccagttaatagtttgcgcaacgttggtgcc  
5601 attgctacaggcatcggtggtgtcacgctcgtcgtttggtatggcttcatt  
5651 cagctccggttcccaacgatcaaggcgagttacatgatcccccatggtgt  
5701 gcaaaaaagcggtagctccttcggctcctccgatcgttgtcagaagtaag  
5751 ttggccgcagtggttatcactcatgggttatggcagcactgcataattctct  
5801 tactgtcatgccatccgtaagatgcttttctgtgactgggtgagtactcaa

FIGURE 8F

5851 ccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccg  
5901 gcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgct  
5951 catcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgc  
6001 tgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttca  
6051 gcatctttttactttcaccagcgtttctgggtgagcaaaaacaggaaggca  
6101 aaatgccgcaaaaaaggggaataagggcgacacggaaatgttgaatactca  
6151 tactcttcctttttcaatattattgaagcatttatcagggttattgtctc  
6201 atgagcggatacatatttgaatgtatttagaaaaataaacaatataggggt  
6251 tccgcgcacatttccccgaaaagtgccacct

FIGURE 9A

1 gacgtcgcggccgctctagggcctccaaaaagcctcctcactacttctg  
51 aatagctcagaggccgaggcgccctcggcctctgcataaataaaaaaat  
101 tagtcagccatgcatggggcggagaatgggcggaactgggcggagttagg  
151 ggcgggatgggcggagttaggggcgggactatggttgctgactaattgag  
201 atgcatgctttgcatacttctgcctgctggggagcctggggactttccac  
251 acctggttgctgactaattgagatgcatgctttgcatacttctgcctgct  
301 ggggagcctggggactttccacaccctaactgacacacattccacagaat  
351 taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat  
401 caattacggggtcattagttcatagcccatatatggagttccgcgttaca  
451 taacttacggtaaatggcccgcctggctgaccgccaacgacccccgccc  
501 attgacgtcaataatgacgtatggtcccatagtaacgccaatagggactt  
551 tccattgacgtcaatgggtggactatttacggtaaaactgcccacttgga  
601 gtacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaatga  
651 cggtaaatggccccgcctggcattatgcccagtacatgaccttatgggact  
701 ttctacttggcagtacatctacgtattagtcacgctattaccatgggtg  
751 atgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacg  
801 gggatttccaagtctccaccccatgacgtcaatgggagtttggtttggc  
851 accaaaatcaacgggactttccaaaatgtcgtacaactccgccccattg  
901 acgcaaattgggcggtaggcgtgtacgggtgggaggtctatataagcagagc  
  
EcoRI  
951 tgggtacgtgaaccgtcagatcgccctggagacgccatcgaattctgagca  
1001 cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca  
M G W S C I I L F L V A  
Leader start  
  
SacI  
1051 acagctacaggtgtccactccgagctcacgcagccgcctcagtctctgc  
T A T G V H S E L T Q --  
Processed N-term

FIGURE 9B

1101 ggccccaggacagaagggtcaccatctcctgcactgggagcagctccaacc  
1151 tcgggggcagggttatgatgttcactgggtaccggcaacttccagggacagcc  
1201 cccaaactcctcatctatgataacaacaatcggccctcaggggtcctga  
1251 ccgattctctggctccaagtctggccccctcagcctccctggccatctctg  
1301 ggctccagggtgaggatgaggctgattattactgccagtcctatgacagc  
1351 agcctgaatgggttatgtcttcggaactgggaccagctcacggtcctagg  
AvrII  
T Q L T V L G  
Framework IV / Cλ  
1401 tcagcccaaggctgccccctcggtcactctgttcccgcctcctctgagg  
1451 agcttcaagccaacaaggccacactgggtgtgtctcataagtgacttctac  
1501 ccgggagccgtgacagtggcctggaaggcaattagcagccccgtcaaggc  
1551 gggagtggagaccaccacaccctccaaacaaagcaacaacaagtacgcgg  
1601 ccagcagctatctgagcctgacgcctgagcagtgggaagtcccacagaagg  
1651 tacagctgccagggtcacgcatgaaggggagcacctggagaagacagtggc  
1701 ccctacagaatgttcatagtttctagatctacgtatgatcagcctcgactg  
P T E C S \* C-term light chain  
1751 tgccttctagttgccagccatctgttggttgccccctccccctgccttcc  
1801 ttgaccctggaagggtgccactcccactgtcctttcctaataaaaatgagga  
1851 aattgcatcgcattgtctgagtaggtgtcattctattctgggggggtgggg  
1901 tggggcaggacagcaagggggaggattgggaagacaatagcaggcatgct  
1951 ggggatgcggtgggctctatggaaccagctggggctcgacagctcgagct  
2001 agcttttgcttctcaatttcttatttgcataatgagaaaaaaaggaaaatt  
2051 aattttaacaccaattcagtagttgattgagcaaatgcgttgccaaaaag  
2101 gatgcttttagagacagtgttctctgcacagataaggacaaacattattca  
2151 gagggagtaccagagctgagactcctaagccagtgagtggcacagcatt

FIGURE 9C

2201 ctagggagaaatatgcttgtcatcaccgaagcctgattccgtagagccac  
2251 accttggtaagggccaatctgctcacacaggatagagaggggcaggagcca  
2301 gggcagagcatataaggtgaggtaggatcagttgctcctcacatttgctt  
2351 ctgacatagttgtgttgggagcttggatcgatccaccatgggttgaacaag  
2401 atggattgcacgcaggttctccggccgcttgggtggagaggctattcggc  
2451 tatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccg  
2501 gctgtcagcgcagggggcgcccggttctttttgtcaagaccgacctgtccg  
2551 gtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggcc  
2601 acgacggggcggttcccttgccgagctgtgctcgacgttgtcactgaagcggg  
2651 aagggactggctgctattgggcgaagtgccggggcaggatctcctgtcat  
2701 ctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgcgg  
2751 cggctgcatacgccttgatccggctacctgcccattcgaccaccaagcgaa  
2801 acatcgcatcgagcgagcacgtactcggatggaagccgggtcttgtcgatc  
2851 aggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttc  
2901 gccaggctcaaggcgcgcatgcccgacggcgaggatctcgtcgtgaccca  
2951 tggcgatgcctgcttgccgaatatcatgggtggaaaatggccgcttttctg  
3001 gattcatcgactgtggccggctgggtgtggcggaccgctatcaggacata  
3051 gcgttggctaccctgtgatattgctgaagagcttggcggcgaatgggctga  
3101 ccgcttcctcgtgctttacggtatcgccgctcccgattcgcagcgcacg  
3151 ccttctatcgccttcttgacgagttcttctgagcgggactctgggggttcg  
3201 aatgaccgaccaagcgacgcccacctgccatcacgagatttcgattcc  
3251 accgccgccttctatgaaagggttgggcttcggaatcgttttccgggacgc  
3301 cggctggatgatcctccagcgcggggatctcatgctggagttcttcgccc  
3351 accccaacttgtttattgcagcttataatgggttacaataaagcaatagc

FIGURE 9D

3401 atcacaaatttcacaaataaagcatttttttttactgcattctagttgtgg  
3451 tttgtccaaactcatcaatgtatcttatcatgtctggatcgcgggccgcga  
3501 tcccgtcgagagcttggcgtaatcatggtcataagctgtttcctgtgtgaa  
3551 attgttatccgctcacaaattccacacaacatacgagccggaagcataaag  
3601 tgtaaagcctgggggtgcctaataagtgagtgagctaactcacattaattgcgtt  
3651 ggcgtcactgcccgcctttccagtcgggaaacctgtcgtgccagctgcatt  
3701 aatgaatcggccaacgcgcggggagaggcggttttgcgtattgggcgctct  
3751 tccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcg  
3801 agcggtatcagctcactcaaaggcggtaatacggttatccacagaatcag  
3851 gggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccagg  
3901 aaccgtaaaaaggccgcgttgctggcggtttttccataggctccgcccccc  
3951 tgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaaccgga  
4001 caggactataaagataaccaggcgtttccccctggaagctccctcgtgcgc  
4051 tctcctgttcgaccctgccgcttaccggatacctgtccgcctttctccc  
4101 ttcgggaagcgtggcgcttttctcaatgctcacgctgtaggtatctcagtt  
4151 cgggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccggt  
4201 cagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaaccc  
4251 ggtaagacacgacttatcgccactggcagcagccactggtaacaggatta  
4301 gcagagcgaggtatgtaggcggtgctacagagttcttgaagtgggtggcct  
4351 aactacggctacactagaaggacagtatttggtatctgcgctctgctgaa  
4401 gccagttaccttcggaaaaagagttggttagctcttgatccggcaaaacaaa  
4451 ccaccgctggtagcgggtgggttttttttgtttgcaagcagcagattacgcgc  
4501 agaaaaaaaggatctcaagaagatcctttgatcttttctacgggggtctga  
4551 cgctcagtggaaacgaaaactcacgttaagggatttttggtcatgagattat

FIGURE 9E

4601 caaaaaggatcttcacctagatccttttaaattaaaaatgaagttttaaa  
4651 tcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgctt  
4701 aatcagtgaggcacctatctcagcgatctgtctatttcgttcacccatag  
4751 ttgcctgactccccgctcgtgtagataactacgatacgggagggccttacca  
4801 tctggccccagtgctgcaatgataccgcgagacccacgctcaccggctcc  
4851 agatttatcagcaataaaccagccagccggaagggccgagcgcagaagtg  
4901 gtccctgcaactttatccgcctccatccagtctattaattggttgccgggaa  
4951 gctagagtaagtagttcgccagttaatagtttgcgcaacgttggtgccat  
5001 tgctacaggcatcgtggtgtcacgctcgtcgtttgggtatggcttcattca  
5051 gctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgc  
5101 aaaaaagcgggttagctccttcggtcctccgatcgttgtcagaagtaagtt  
5151 ggccgcagtggttatcactcatgggttatggcagcactgcataattctctta  
5201 ctgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaacc  
5251 aagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggc  
5301 gtcaatacgggataataccgcgccacatagcagaactttaaaagtgtca  
5351 tcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctg  
5401 ttgagatccagttcgatgtaaccactcgtgcacccaactgatcttcagc  
5451 atcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaa  
5501 atgccgcaaaaaagggaataagggcgacacggaaatggttgaatactcata  
5551 ctcttcctttttcaatattattgaagcatttatcagggttattgtctcat  
5601 gagcggatacatatttgatgtatttagaaaaataaacaatataggggttc  
5651 cgcgcacatttccccgaaaagtgccacct

FIGURE 10A

	EcoRI <u>gaattctgagca</u>	1000
cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca		1050
M G W S C I I L F L V A		
acagctacaggtgtccactccgaggtgcagctggaggagtcctgggggagg		1100
T A T G V H S <u>E V Q</u> L <u>V</u> E S -		
N-term		
cttgggtacagcctgggggggtccctgagactctcctgcgagcctctggag		1150
tctccctcagtggtatacaagatgaactgggtccgccaggctccagggaag		1200
gggctggaatgggtctcttccattactgggtatgagtaattacatacacta		1250
ctcagactcagtggaagggccgattcaccatctccagagacaacgccatga		1300
actcactgtatctgcaaataaacagcctgacagccgaggacacgggtgtt		1350
tattattgtgagacacaaccgggggagctggcgcccttttgaccattgggg		1400
	Bsp120I	
ccagggaaccctgggtcacggtctcctcagcctccaccaagggcccatcg		1450
tcttccccctggcacccctcctccaagagcacctctgggggacagcgcc		1500
ctgggctgcctgggtcaaggactacttccccgaaccgggtgacgggtgtcgtg		1550
gaactcaggcgccctgaccagcggcggtgcacaccttcccggctgtcctac		1600
agtcctcaggactctactccctcagcagcgtgggtgaccgtgcctccagc		1650
agcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaa		1700
caccaaggtggacaagaaagttgagcccaaatacttgtagacaaaactcaca		1750
catgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttc		1800
ctcttccccccaaaacccaaggacaccctcatgatctcccgaccctga		1850
ggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagt		1900
tcaactgggtacgtggacggcggtggaggtgcataatgccaagacaaagccg		1950
cgggaggagcagtagacaacagcacgtaccgggtgggtcagcgtcctcaccgt		2000
cctgcaccaggactgggtgaatggcaaggagtacaagtgcaaggtctcca		2050

FIGURE 10B

acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggg	2100
cagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagct	2150
gaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccca	2200
gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac	2250
aagaccacgcctcccgtgctggactccgacggctccttcttctctacag	2300
caagctcacctggacaagagcaggtggcagcaggggaacgtcttctcat	2350
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc	2400
tcctgtctccgggtaaatgatagatatct	
S  P  G  K  *	

FIGURE 11

	EcoRI	
	<u>gaattctgagca</u>	1000
cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca		1050
M G W S C I I L F L V A		
acagctacaggtgtccactcc <u>cagtctgtgtg</u> acgcagccgccctcagt		1100
T A T G V H S <u>Q S V</u> L T Q -		
	N-term	
ctctgcggccccaggacagaagggtcaccatctcctgcactgggagcagct		1150
ccaacctcggggcagggttatgatgttcactgggtaccggcaacttccaggg		1200
acagcccccaactcctcatctatgataacaacaatcggccctcaggggt		1250
ccctgaccgattctctggctccaagtctggccccctcagcctccctggcca		1300
tctctgggctccaggtgaggatgaggctgattattactgccagtcctat		1350
gacagcagcctgaatgggttatgtcttcggaactgggaccagctcacgt		1400
AvrII		
<u>cctagg</u> tcagcccaaggctgccccctcggtcactctgttcccgcctcct		1450
ctgaggagcttcaagccaacaaggccacactgggtgtgtctcataagtgac		1500
ttctaccggggagccgtgacagtggcctggaaggcaattagcagccccgt		1550
caaggcgggagtgagagaccaccacaccctccaaacaagcaacaacaagt		1600
acgcggccagcagctatctgagcctgacgcctgagcagtggaagtccac		1650
agaaggtacagctgccaggtcacgcatgaaggagcaccgtggagaagac		1700
agtggccccctacagaatgttc <u>atag</u> ttctagatctacgtatgatcagcct		1750
P T E C S *		

# SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: SmithKline Beecham, PLC
- (ii) TITLE OF INVENTION: Human Monoclonal Antibody
- (iii) NUMBER OF SEQUENCES: 21
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: SmithKline Beecham Corporation
  - (B) STREET: 709 Swedeland Road
  - (C) CITY: King of Prussia
  - (D) STATE: PA
  - (E) COUNTRY: USA
  - (F) ZIP: 19406-2799
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: GB
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: King, William T.
  - (B) REGISTRATION NUMBER: 30,954
  - (C) REFERENCE/DOCKET NUMBER: #
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 610-270-4800
  - (B) TELEFAX: 610-270-4026

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 336 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

```
(ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 1..336
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAG TCT GTG TTG ACG CAG CCG CCC TCA GTC TCT GCG GCC CCA GGA CAG 48  
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln  
1 5 10 15

AAG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC CTC GGG GCA GGT 96

Lys	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser	Ser	Ser	Asn	Leu	Gly	Ala	Gly		
			20					25					30				
TAT	GAT	GTT	CAC	TGG	TAC	CGG	CAA	CTT	CCA	GGG	ACA	GCC	CCC	AAA	CTC		144
Tyr	Asp	Val	His	Trp	Tyr	Arg	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu		
		35					40					45					
CTC	ATC	TAT	GAT	AAC	AAC	AAT	CGG	CCC	TCA	GGG	GTC	CCT	GAC	CGA	TTC		192
Leu	Ile	Tyr	Asp	Asn	Asn	Asn	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe		
	50					55					60						
TCT	GGC	TCC	AAG	TCT	GGC	CCC	TCA	GCC	TCC	CTG	GCC	ATC	TCT	GGG	CTC		240
Ser	Gly	Ser	Lys	Ser		70	Pro	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu	
65										75					80		
CAG	GCT	GAG	GAT	GAG	GCT	GAT	TAT	TAC	TGC	CAG	TCC	TAT	GAC	AGC	AGC		288
Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Ser	Ser		
			85						90					95			
CTG	AAT	GGT	TAT	GTC	TTC	GGA	ACT	GGG	ACC	CAG	CTC	ACC	GTC	CTA	GGT		336
Leu	Asn	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Gln	Leu	Thr	Val	Leu	Gly		
		100						105					110				

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Ala	Ala	Pro	Gly	Gln		
1				5					10					15			
Lys	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser	Ser	Ser	Asn	Leu	Gly	Ala	Gly		
		20						25					30				
Tyr	Asp	Val	His	Trp	Tyr	Arg	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu		
	35						40					45					
Leu	Ile	Tyr	Asp	Asn	Asn	Asn	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe		
	50					55					60						
Ser	Gly	Ser	Lys	Ser	Gly	Pro	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu		
65					70					75					80		
Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Ser	Ser		
			85						90					95			
Leu	Asn	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Gln	Leu	Thr	Val	Leu	Gly		
		100						105					110				

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG	48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
TCC CTG AGA CTC TCC TGC GCA GCC TCT GGA GTC TCC CTC AGT GGA TAC	96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr	
20 25 30	
AAG ATG AAC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAA TGG GTC	144
Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
TCT TCC ATT ACT GGT ATG AGT AAT TAC ATA CAC TAC TCA GAC TCA GTG	192
Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser Asp Ser Val	
50 55 60	
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC ATG AAC TCA CTG TAT	240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn Ser Leu Tyr	
65 70 75 80	
CTG CAA ATG AAC AGC CTG ACA GCC GAG GAC ACG GGT GTT TAT TAT TGT	288
Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val Tyr Tyr Cys	
85 90 95	
GCG ACA CAA CCG GGG GAG CTG GCG CCT TTT GAC CAT TGG GGC CAG GGA	336
Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp Gly Gln Gly	
100 105 110	
ACC CTG GTC ACC GTC TCC TCA	357
Thr Leu Val Thr Val Ser Ser	
115	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr
20 25 30
Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35						40					45				
Ser	Ser	Ile	Thr	Gly	Met	Ser	Asn	Tyr	Ile	His	Tyr	Ser	Asp	Ser	Val
	50					55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Met	Asn	Ser	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Thr	Ala	Glu	Asp	Thr	Gly	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Thr	Gln	Pro	Gly	Glu	Leu	Ala	Pro	Phe	Asp	His	Trp	Gly	Gln	Gly
			100					105					110		
Thr	Leu	Val	Thr	Val	Ser	Ser									
		115													

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

[illegible]

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20           25           30
Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35           40           45
Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50           55           60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65           70           75           80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85           90           95
Ala Arg

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 138 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1           5           10           15
Val His Ser Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
20           25           30
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu
35           40           45
Ser Gly Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50           55           60
Glu Trp Val Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser
65           70           75           80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn
85           90           95
Ser Leu Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val

```

100							105				110				
Tyr	Tyr	Cys	Ala	Thr	Gln	Pro	Gly	Glu	Leu	Ala	Pro	Phe	Asp	His	Trp
115							120				125				
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser						
130						135									

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 138 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met 1	Gly	Trp	Ser	Cys 5	Ile	Ile	Leu	Phe	Leu 10	Val	Ala	Thr	Ala	Thr 15	Gly
Val	His	Ser	Glu 20	Val	Gln	Leu	Val	Glu 25	Ser	Gly	Gly	Gly	Leu 30	Val	Gln
Pro	Gly	Gly 35	Ser	Leu	Arg	Leu	Ser 40	Cys	Ala	Ala	Ser	Gly 45	Val	Ser	Leu
Ser	Gly 50	Tyr	Lys	Met	Asn	Trp 55	Val	Arg	Gln	Ala	Pro 60	Gly	Lys	Gly	Leu
Glu 65	Trp	Val	Ser	Ser	Ile 70	Thr	Gly	Met	Ser	Asn 75	Tyr	Ile	His	Tyr	Ser 80
Asp	Ser	Val	Lys	Gly 85	Arg	Phe	Thr	Ile	Ser 90	Arg	Asp	Asn	Ala	Met 95	Asn
Ser	Leu	Tyr	Leu 100	Gln	Met	Asn	Ser	Leu 105	Thr	Ala	Glu	Asp	Thr 110	Gly	Val
Tyr	Tyr	Cys 115	Ala	Thr	Gln	Pro	Gly 120	Glu	Leu	Ala	Pro	Phe 125	Asp	His	Trp
Gly 130	Gln	Gly	Thr	Leu	Val	Thr 135	Val	Ser	Ser						

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1             5             10             15
Lys Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Leu Gly Ala Gly
          20             25             30
Tyr Asp Val His Trp Tyr Arg Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35             40             45
Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
          50             55             60
Ser Gly Ser Lys Ser Gly Pro Ser Ala Ser Leu Ala Ile Ser Gly Leu
          65             70             75             80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
          85             90             95
Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
          100            105            110

```

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 90 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1             5             10             15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
          20             25             30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35             40             45
Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
          50             55             60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
          65             70             75             80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys
          85             90

```

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 128 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Glu Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly
          20           25           30
Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala
          35           40           45
Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
          50           55           60
Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg
          65           70           75           80
Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly
          85           90           95
Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
          100          105          110
Ser Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
          115          120          125

```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 130 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala
          20           25           30
Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile
          35           40           45
Gly Ala Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala
          50           55           60
Pro Lys Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro
          65           70           75           80

```

Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile  
                                     85                                    90                                    95  
 Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr  
                                     100                                    105                                    110  
 Asp Ser Ser Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr  
                                     115                                    120                                    125  
 Val Leu  
                     130

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 6281 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG	60
AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAT TAGTCAGCCA TGCATGGGGC	120
GGAGAATGGG CGGAAGTGGG CGGAGTTAGG GCGGGGATGG GCGGAGTTAG GGGCGGGACT	180
ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG	240
GACTTTCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT	300
GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG	360
GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT	420
CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACCTACGG TAAATGGCCC GCCTGGCTGA	480
CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA	540
ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGA	600
GTACATCAAG TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG	660
CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC	720
TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT	780
GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT	840
TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAACAACTC CGCCCCATTG	900
ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG	960
AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCTGAGCA CACAGGACCT CACCATGGGA	1020
TGGAGCTGTA TCATCCTCTT CTTGGTAGCA ACAGCTACAG GTGTCCACTC CGAGGTCCAA	1080

CTGCTCGAGT	CTGGGGGAGG	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGCGCA	1140
GCCTCTGGAG	TCTCCCTCAG	TGGATACAAG	ATGAACTGGG	TCCGCCAGGC	TCCAGGGAAG	1200
GGGCTGGAAT	GGGTCTCTTC	CATTACTGGT	ATGAGTAATT	ACATACACTA	CTCAGACTCA	1260
GTGAAGGGCC	GATTCAACCAT	CTCCAGAGAC	AACGCCATGA	ACTCACTGTA	TCTGCAAATG	1320
AACAGCCTGA	CAGCCGAGGA	CACGGGTGTT	TATTATTGTG	CGACACAACC	GGGGGAGCTG	1380
GCGCCTTTTG	ACCATTGGGG	CCAGGGAACC	CTGGTCACCG	TCTCCTCAGC	CTCCACCAAG	1440
GGCCCATCGG	TCTTCCCCCT	GGCACCCCTC	TCCAAGAGCA	CCTCTGGGGG	CACAGCGGCC	1500
CTGGGCTGCC	TGGTCAAGGA	CTACTTCCCC	GAACCGGTGA	CGGTGTCGTG	GAACTCAGGC	1560
GCCCTGACCA	GCGGCGTGCA	CACCTTCCCG	GCTGTCTTAC	AGTCCTCAGG	ACTCTACTCC	1620
CTCAGCAGCG	TGGTGACCGT	GCCCTCCAGC	AGCTTGGGCA	CCCAGACCTA	CATCTGCAAC	1680
GTGAATCACA	AGCCCAGCAA	CACCAAGGTG	GACAAGAAAG	TTGAGCCCAA	ATCTTGTGAC	1740
AAAACTCACA	CATGCCCACC	GTGCCCAGCA	CCTGAACTCC	TGGGGGGACC	GTCAGTCTTC	1800
CTCTTCCCCC	CAAAACCCAA	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	1860
GTGGTGGTGG	ACGTGAGCCA	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	1920
GTGGAGGTGC	ATAATGCCAA	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGG	1980
GTGGTCAGCG	TCCTCACCGT	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	2040
AAGGTCTCCA	ACAAAGCCCT	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGG	2100
CAGCCCCGAG	AACCACAGGT	GTACACCCTG	CCCCCATCCC	GGGATGAGCT	GACCAAGAAC	2160
CAGGTCAGCC	TGACCTGCCT	GGTCAAAGGC	TTCTATCCCA	GCGACATCGC	CGTGGAGTGG	2220
GAGAGCAATG	GGCAGCCGGA	GAACAACCTAC	AAGACCACGC	CTCCCGTGCT	GGA CTCCGAC	2280
GGCTCCTTCT	TCCTCTACAG	CAAGCTCACC	GTGGACAAGA	GCAGGTGGCA	GCAGGGGAAC	2340
GTCTTCTCAT	GCTCCGTGAT	GCATGAGGCT	CTGCACAACC	ACTACACGCA	GAAGAGCCTC	2400
TCCCTGTCTC	CGGGTAAATG	ATAGATATCT	ACGTATGATC	AGCCTCGACT	GTGCCTTCTA	2460
GTTGCCAGCC	ATCTGTTGTT	TGCCCCCTCC	CCGTGCCTTC	CTTGACCCTG	GAAGGTGCCA	2520
CTCCCACTGT	CCTTTCCTAA	TAAAATGAGG	AAATTGCATC	GCATTGTCTG	AGTAGGTGTC	2580
ATTCTATTCT	GGGGGGTGGG	GTGGGGCAGG	ACAGCAAGGG	GGAGGATTGG	GAAGACAATA	2640
GCAGGCATGC	TGGGGATGCG	GTGGGCTCTA	TGGAACCAGC	TGGGGCTCGA	CAGCGCTGGA	2700
TCTCCCGATC	CCCAGCTTTG	CTTCTCAATT	TCTTATTTGC	ATAATGAGAA	AAAAAGGAAA	2760
ATTAATTTTA	ACACCAATTC	AGTAGTTGAT	TGAGCAAATG	CGTTGCCAAA	AAGGATGCTT	2820
TAGAGACAGT	GTTCTCTGCA	CAGATAAGGA	CAAACATTAT	TCAGAGGGAG	TACCCAGAGC	2880
TGAGACTCCT	AAGCCAGTGA	GTGGCACAGC	ATTCTAGGGA	GAAATATGCT	TGTCATCACC	2940
GAAGCCTGAT	TCCGTAGAGC	CACACCTTGG	TAAGGGCCAA	TCTGCTCACA	CAGGATAGAG	3000

AGGGCAGGAG	CCAGGGCAGA	GCATATAAGG	TGAGGTAGGA	TCAGTTGCTC	CTCACATTTG	3060
CTTCTGACAT	AGTTGTGTTG	GGAGCTTGGA	TAGCTTGGAC	AGCTCAGGGC	TGCGATTTTCG	3120
CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	3180
ATGGTTCGAC	CATTGAACTG	CATCGTCGCC	GTGTCCCAAA	ATATGGGGAT	TGGCAAGAAC	3240
GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAAGT	ACTTCCAAAG	AATGACCACA	3300
ACCTCTTCAG	TGGAAGGTAA	ACAGAATCTG	GTGATTATGG	GTAGGAAAAC	CTGGTTCTCC	3360
ATTCTTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	3420
AAAGAACCAC	CACGAGGAGC	TCATTTTCTT	GCCAAAAGTT	TGGATGATGC	CTTAAGACTT	3480
ATTGAACAAC	CGGAATTGGC	AAGTAAAGTA	GACATGGTTT	GGATAGTCGG	AGGCAGTTCT	3540
GTTTACCAGG	AAGCCATGAA	TCAACCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	3600
CAGGAATTTG	AAAGTGACAC	GTTTTTCCCA	GAAATTGATT	TGGGGAAATA	TAAACTTCTC	3660
CCAGAATACC	CAGGCGTCCT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	3720
GAAGTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	TCCCCTCCTA	3780
AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTGTCTGGC	TTTAGATCAG	CCTCGACTGT	3840
GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTG	CCCCTCCCCC	GTGCCTTCCT	TGACCCCTGGA	3900
AGGTGCCACT	CCCCTGTGCC	TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGTCTGAG	3960
TAGGTGTCAT	TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	4020
AGACAATAGC	AGGCATGCTG	GGGATGCGGT	GGGCTCTATG	GAACCAGCTG	GGGCTCGATC	4080
GAGTGTATGA	CTGCGGCCGC	GATCCCGTCG	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	4140
TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC	GGAAGCATAA	4200
AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	4260
TGCCCCGCTTT	CCAGTCGGGA	AACCTGTTCG	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	4320
CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	4380
GCTCGGTTCG	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	4440
CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	4500
GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	4560
ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	GACAGGACTA	TAAAGATACC	4620
AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	4680
GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA	4740
GGTATCTCAG	TTCGGTGTAG	GTCGTTGCTC	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	4800
TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	4860

ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	4920
GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	4980
TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	5040
CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC	5100
GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	5160
GGAACGAAAA	CTCACGTAA	GGGATTTTGG	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	5220
AGATCCTTTT	AAATTAAAAA	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	5280
GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTT	5340
GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCT	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	5400
CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG	CTCACC GGCT	CCAGATTTAT	5460
CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	5520
CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCT	CCAGTTAATA	5580
GTTTGCGCAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA	5640
TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	5700
GCAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	5760
TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	5820
GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	5880
GACCGAGTTG	CTCTTGCCCC	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	5940
TAAAAGTGCT	CATCATTTGA	AAACGTTCTT	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	6000
TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	6060
CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	6120
TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	6180
TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	ATGTATTTAG	AAAAATAAAC	6240
AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	T		6281

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GACGTCGCGG	CCGCTCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	60
AGGCCGAGGC	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAT	TAGTCAGCCA	TGCATGGGGC	120
GGAGAATGGG	CGGAACTGGG	CGGAGTTAGG	GGCGGGATGG	GCGGAGTTAG	GGGCGGGACT	180
ATGGTTGCTG	ACTAATTGAG	ATGCATGCTT	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	240
GACTTTCCAC	ACCTGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCATACT	TCTGCCTGCT	300
GGGGAGCCTG	GGGACTTTCC	ACACCCTAAC	TGACACACAT	TCCACAGAAT	TAATTCCCGG	360
GGATCGATCC	GTCGACGTAC	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	420
CATAGCCCAT	ATATGGAGTT	CCGCGTTACA	TAACTTACGG	TAAATGGCCC	GCCTGGCTGA	480
CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	AGTAACGCCA	540
ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTAC	GGTAAACTGC	CCACTTGGCA	600
GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG	660
CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCTACTTG	GCAGTACATC	720
TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	780
GGATAGCGGT	TTGACTCACG	GGGATTTCCA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	840
TTGTTTTGGC	ACCAAATCA	ACGGGACTTT	CCAAAATGTC	GTAACAAC TC	CGCCCCATTG	900
ACGCAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	960
AACCGTCAGA	TCGCCTGGAG	ACGCCATCGA	ATTCTGAGCA	CACAGGACCT	CACCATGGGA	1020
TGGAGCTGTA	TCATCCTCTT	CTTGGTAGCA	ACAGCTACAG	GTGTCCACTC	CGAGCTCACG	1080
CAGCCGCCCT	CAGTCTCTGC	GGCCCCAGGA	CAGAAGGTCA	CCATCTCCTG	CACTGGGAGC	1140
AGCTCCAACC	TCGGGGCAGG	TTATGATGTT	CACTGGTACC	GGCAACTTCC	AGGGACAGCC	1200
CCCAAAC TCC	TCATCTATGA	TAACAACAAT	CGGCCCTCAG	GGGTCCCTGA	CCGATTCTCT	1260
GGCTCCAAGT	CTGGCCCCCTC	AGCCTCCCTG	GCCATCTCTG	GGCTCCAGGC	TGAGGATGAG	1320
GCTGATTATT	ACTGCCAGTC	CTATGACAGC	AGCCTGAATG	GTTATGTCTT	CGGAACTGGG	1380
ACCCAGCTCA	CCGTCC TAGG	TCAGCCCCAAG	GCTGCCCCCT	CGGTCACTCT	GTTCCCGCCC	1440
TCCTCTGAGG	AGCTTCAAGC	CAACAAGGCC	ACACTGGTGT	GTCTCATAAG	TGACTTCTAC	1500
CCGGGAGCCG	TGACAGTGGC	CTGGAAGGCA	ATTAGCAGCC	CCGTCAAGGC	GGGAGTGGAG	1560
ACCACCACAC	CCTCCAAACA	AAGCAACAAC	AAGTACGCGG	CCAGCAGCTA	TCTGAGCCTG	1620
ACGCCTGAGC	AGTGGAAGTC	CCACAGAAGG	TACAGCTGCC	AGGTCACGCA	TGAAGGGAGC	1680
ACCGTGGAGA	AGACAGTGGC	CCCTACAGAA	TGTTCATAGT	TCTAGATCTA	CGTATGATCA	1740
GCCTCGACTG	TGCCTTCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCCTCCC	CGTGCCTTCC	1800
TTGACCCTGG	AAGGTGCCAC	TCCC ACTGTC	CTTTCCTAAT	AAAATGAGGA	AATTGCATCG	1860
CATTGTCTGA	GTAGGTGTCA	TTCTATTCTG	GGGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	1920

GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGAACCAGCT	1980
GGGGCTCGAC	AGCTCGAGCT	AGCTTTGCTT	CTCAATTTCT	TATTTGCATA	ATGAGAAAAA	2040
AAGGAAAATT	AATTTTAACA	CCAATTCAGT	AGTTGATTGA	GCAAATGCGT	TGCCAAAAAG	2100
GATGCTTTAG	AGACAGTGTT	CTCTGCACAG	ATAAGGACAA	ACATTATTCA	GAGGGAGTAC	2160
CCAGAGCTGA	GACTCCTAAG	CCAGTGAGTG	GCACAGCATT	CTAGGGAGAA	ATATGCTTGT	2220
CATCACCGAA	GCCTGATTCC	GTAGAGCCAC	ACCTTGGTAA	GGGCCAATCT	GCTCACACAG	2280
GATAGAGAGG	GCAGGAGCCA	GGGCAGAGCA	TATAAGGTGA	GGTAGGATCA	GTTGCTCCTC	2340
ACATTTGCTT	CTGACATAGT	TGTGTTGGGA	GCTTGGATCG	ATCCACCATG	GTTGAACAAG	2400
ATGGATTGCA	CGCAGGTTCT	CCGGCCGCTT	GGGTGGAGAG	GCTATTTCGGC	TATGACTGGG	2460
CACAACAGAC	AATCGGCTGC	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	CAGGGGCGCC	2520
CGGTTCTTTT	TGTCAAGACC	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	GACGAGGCAG	2580
CGCGGCTATC	GTGGCTGGCC	ACGACGGGCG	TTCCTTGCGC	AGCTGTGCTC	GACGTTGTCA	2640
CTGAAGCGGG	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	CTCCTGTCAT	2700
CTCACCTTGC	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	CGGCTGCATA	2760
CGCTTGATCC	GGCTACCTGC	CCATTTCGACC	ACCAAGCGAA	ACATCGCATC	GAGCGAGCAC	2820
GTACTIONGAT	GGAAGCCGGT	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	CATCAGGGGC	2880
TCGCGCCAGC	CGAACTGTTC	GCCAGGCTCA	AGGCGCGCAT	GCCCGACGGC	GAGGATCTCG	2940
TCGTGACCCA	TGGCGATGCC	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	CGCTTTTCTG	3000
GATTCATCGA	CTGTGGCCGG	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	GCGTTGGCTA	3060
CCCGTGATAT	TGCTGAAGAG	CTTGGCGGCG	AATGGGCTGA	CCGCTTCCTC	GTGCTTTACG	3120
GTATCGCCGC	TCCCATTTCG	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	GAGTTCTTCT	3180
GAGCGGGACT	CTGGGGTTTCG	AAATGACCGA	CCAAGCGACG	CCCAACCTGC	CATCACGAGA	3240
TTTCGATTCC	ACCGCCGCCT	TCTATGAAAG	GTTGGGCTTC	GGAATCGTTT	TCCGGGACGC	3300
CGGCTGGATG	ATCCTCCAGC	GCGGGGATCT	CATGCTGGAG	TTCTTCGCCC	ACCCCAACTT	3360
GTTTATTGCA	GCTTATAATG	GTTACAAATA	AAGCAATAGC	ATCACAAATT	TCACAAATAA	3420
AGCATTTTTT	TACTGCATT	CTAGTTGTGG	TTTGTCCAAA	CTCATCAATG	TATCTTATCA	3480
TGTCTGGATC	GCGGCCGCGA	TCCCGTCGAG	AGCTTGGCGT	AATCATGGTC	ATAGCTGTTT	3540
CCTGTGTGAA	ATTGTTATCC	GCTCACAATT	CCACACAACA	TACGAGCCGG	AAGCATAAAG	3600
TGTAAAGCCT	GGGGTGCCTA	ATGAGTGAGC	TAATCAGCAT	TAATTGCGTT	GCGCTCACTG	3660
CCCGCTTTCC	AGTCGGGAAA	CCTGTCTGTC	CAGCTGCATT	AATGAATCGG	CCAACGCGCG	3720
GGGAGAGGCG	GTTTGCGTAT	TGGGCGCTCT	TCCGCTTCCT	CGCTCACTGA	CTCGCTGCGC	3780

TCGGTCGTTT	GGCTGCGGCG	AGCGGTATCA	GCTCACTCAA	AGGCGGTAAT	ACGGTTATCC	3840
ACAGAATCAG	GGGATAACGC	AGGAAAGAAC	ATGTGAGCAA	AAGGCCAGCA	AAAGGCCAGG	3900
AACCGTAAAA	AGGCCGCGTT	GCTGGCGTTT	TTCCATAGGC	TCCGCCCCCC	TGACGAGCAT	3960
CACAAAAATC	GACGCTCAAG	TCAGAGGTGG	CGAAACCCGA	CAGGACTATA	AAGATACCAG	4020
GCGTTTCCCC	CTGGAAGCTC	CCTCGTGCGC	TCTCCTGTTC	CGACCCTGCC	GCTTACCGGA	4080
TACCTGTCCG	CCTTTCTCCC	TTCGGGAAGC	GTGGCGCTTT	CTCAATGCTC	ACGCTGTAGG	4140
TATCTCAGTT	CGGTGTAGGT	CGTTCGCTCC	AAGCTGGGCT	GTGTGCACGA	ACCCCCCGTT	4200
CAGCCCGACC	GCTGCGCCTT	ATCCGGTAAC	TATCGTCTTG	AGTCCAACCC	GGTAAGACAC	4260
GACTTATCGC	CACTGGCAGC	AGCCACTGGT	AACAGGATTA	GCAGAGCGAG	GTATGTAGGC	4320
GGTGCTACAG	AGTTCTTGAA	GTGGTGGCCT	AACACTACGG	ACACTAGAAG	GACAGTATTT	4380
GGTATCTGCG	CTCTGCTGAA	GCCAGTTACC	TTCGGAAAAA	GAGTTGGTAG	CTCTTGATCC	4440
GGCAAACAAA	CCACCGCTGG	TAGCGGTGGT	TTTTTTGTTT	GCAAGCAGCA	GATTACGCGC	4500
AGAAAAAAAG	GATCTCAAGA	AGATCCTTTG	ATCTTTTCTA	CGGGGTCTGA	CGCTCAGTGG	4560
AACGAAAACT	CACGTTAAGG	GATTTTGGTC	ATGAGATTAT	CAAAAAGGAT	CTTCACCTAG	4620
ATCCTTTTAA	ATTAAAAATG	AAGTTTAAAA	TCAATCTAAA	GTATATATGA	GTAAACTTGG	4680
TCTGACAGTT	ACCAATGCTT	AATCAGTGAG	GCACCTATCT	CAGCGATCTG	TCTATTTCTG	4740
TCATCCATAG	TTGCCTGACT	CCCCGTCGTG	TAGATAACTA	CGATACGGGA	GGGCTTACCA	4800
TCTGGCCCCA	GTGCTGCAAT	GATACCGCGA	GACCCACGCT	CACCGGCTCC	AGATTTATCA	4860
GCAATAAACC	AGCCAGCCGG	AAGGGCCGAG	CGCAGAAGTG	GTCCTGCAAC	TTTATCCGCC	4920
TCCATCCAGT	CTATTAATTG	TTGCCGGGAA	GCTAGAGTAA	GTAGTTCGCC	AGTTAATAGT	4980
TTGCGCAACG	TTGTTGCCAT	TGCTACAGGC	ATCGTGGTGT	CACGCTCGTC	GTTTGGTATG	5040
GCTTCATTCA	GCTCCGGTTC	CCAACGATCA	AGGCGAGTTA	CATGATCCCC	CATGTTGTGC	5100
AAAAAAGCGG	TTAGCTCCTT	CGGTCTCCG	ATCGTTGTCA	GAAGTAAGTT	GGCCGCAGTG	5160
TTATCACTCA	TGGTTATGGC	AGCACTGCAT	AATTCTCTTA	CTGTCATGCC	ATCCGTAAGA	5220
TGCTTTTCTG	TGACTGGTGA	GTACTCAACC	AAGTCATTCT	GAGAATAGTG	TATGCGGCGA	5280
CCGAGTTGCT	CTTGCCCCGC	GTCAATACGG	GATAATACCG	CGCCACATAG	CAGAACTTTA	5340
AAAGTGCTCA	TCATTGGAAA	ACGTTCTTCG	GGGCGAAAAC	TCTCAAGGAT	CTTACCGCTG	5400
TTGAGATCCA	GTTTCGATGA	ACCCACTCGT	GCACCCAAC	GATCTTCAGC	ATCTTTTACT	5460
TTCACCAGCG	TTTCTGGGTG	AGCAAAAACA	GGAAGGCAAA	ATGCCGCAAA	AAAGGGAATA	5520
AGGGCGACAC	GGAAATGTTG	AATACTCATA	CTCTTCCTTT	TTCAATATTA	TTGAAGCATT	5580
TATCAGGGTT	ATTGTCTCAT	GAGCGGATAC	ATATTTGAAT	GTATTTAGAA	AAATAAACAA	5640
ATAGGGGTTC	CGCGCACATT	TCCCCGAAAA	GTGCCACCT			5679

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1442 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG	60
CAACAGCTAC AGGTGTCCAC TCCGAGGTGC AGCTGGTGGA GTCTGGGGGA GGCTTGGTAC	120
AGCCTGGGGG GTCCCTGAGA CTCTCCTGCG CAGCCTCTGG AGTCTCCCTC AGTGGATACA	180
AGATGAACTG GGTCCGCCAG GCTCCAGGGA AGGGGCTGGA ATGGGTCTCT TCCATTACTG	240
GTATGAGTAA TTACATACAC TACTCAGACT CAGTGAAGGG CCGATTACAC ATCTCCAGAG	300
ACAACGCCAT GAACTCACTG TATCTGCAA TGAACAGCCT GACAGCCGAG GACACGGGTG	360
TTTATTATTG TGCGACACAA CCGGGGGAGC TGGCGCCTTT TGACCATTGG GGCCAGGGAA	420
CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT	480
CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC	540
CCGAACCGGT GACGGTGTCTG TGGAATCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC	600
CGGCTGTCTT ACAGTCCTCA GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA	660
GCAGCTTGGG CACCCAGACC TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG	720
TGGACAAGAA AGTTGAGCCC AAATCTTGTG ACAAACCTCA CACATGCCCA CCGTGCCCAG	780
CACCTGAACT CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC	840
TCATGATCTC CCGGACCCCT GAGGTACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC	900
CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC	960
CGCGGGAGGA GCAGTACAAC AGCACGTACC GGGTGGTCAG CGTCCTCACC GTCCTGCACC	1020
AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC	1080
CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC	1140
TGCCCCCATC CCGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG	1200
GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACCT	1260
ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTCCTCTAC AGCAAGCTCA	1320
CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG	1380
CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGATAGATAT	1440

CT

1442

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 762 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG	60
CAACAGCTAC AGGTGTCCAC TCCCAGTCTG TGTTGACGCA GCCGCCCTCA GTCTCTGCGG	120
CCCCAGGACA GAAGGTCACC ATCTCCTGCA CTGGGAGCAG CTCCAACCTC GGGGCAGGTT	180
ATGATGTTCA CTGGTACCGG CAACTTCCAG GGACAGCCCC CAAACTCCTC ATCTATGATA	240
ACAACAATCG GCCCTCAGGG GTCCCTGACC GATTCTCTGG CTCCAAGTCT GGCCCCCTCAG	300
CCTCCCTGGC CATCTCTGGG CTCCAGGCTG AGGATGAGGC TGATTATTAC TGCCAGTCCT	360
ATGACAGCAG CCTGAATGGT TATGTCTTCG GAACTGGGAC CCAGCTCACC GTCCTAGGTC	420
AGCCCAAGGC TGCCCCCTCG GTCACTCTGT TCCCGCCCTC CTCTGAGGAG CTTCAAGCCA	480
ACAAGGCCAC ACTGGTGTGT CTCATAAGTG ACTTCTACCC GGGAGCCGTG ACAGTGGCCT	540
GGAAGGCAAT TAGCAGCCCC GTCAAGGCGG GAGTGGAGAC CACCACACCC TCCAAACAAA	600
GCAACAACAA GTACGCGGCC AGCAGCTATC TGAGCCTGAC GCCTGAGCAG TGGAAGTCCC	660
ACAGAAGGTA CAGCTGCCAG GTCACGCATG AAGGGAGCAC CGTGGAGAAG ACAGTGGCCC	720
CTACAGAATG TTCATAGTTC TAGATCTACG TATGATCAGC CT	762

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu	Val	Gln	Leu	Leu	Glu
1				5	

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Val Gln Leu Val Glu  
 1 5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1899 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 14..1735

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGGCAAATA ACA ATG GAG TTG CTA ATC CTC AAA GCA AAT GCA ATT ACC	49
Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr	
1 5 10	
ACA ATC CTC ACT GCA GTC ACA TTT TGT TTT GCT TCT GGT CAA AAC ATC	97
Thr Ile Leu Thr Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile	
15 20 25	
ACT GAA GAA TTT TAT CAA TCA ACA TGC AGT GCA GTT AGC AAA GGC TAT	145
Thr Glu Glu Phe Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr	
30 35 40	
CTT AGT GCT CTG AGA ACT GGT TGG TAT ACC AGT GTT ATA ACT ATA GAA	193
Leu Ser Ala Leu Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu	
45 50 55 60	
TTA AGT AAT ATC AAG GAA AAT AAG TGT AAT GGA ACA GAT GCT AAG GTA	241
Leu Ser Asn Ile Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val	
65 70 75	
AAA TTG ATA AAA CAA GAA TTA GAT AAA TAT AAA AAT GCT GTA ACA GAA	289
Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu	
80 85 90	
TTG CAG TTG CTC ATG CAA AGC ACA CCA CCA ACA AAC AAT CGA GCC AGA	337
Leu Gln Leu Leu Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg	
95 100 105	

AGA Arg 110	GAA Glu 110	CTA Leu 110	CCA Pro 110	AGG Arg 110	TTT Phe 110	ATG Met 115	AAT Asn 115	TAT Tyr 115	ACA Thr 115	CTC Leu 120	AAC Asn 120	AAT Asn 120	GCC Ala 120	AAA Lys 120	AAA Lys 120	385
ACC Thr 125	AAT Asn 125	GTA Val 125	ACA Thr 125	TTA Leu 130	AGC Ser 130	AAG Lys 130	AAA Lys 130	AGG Arg 135	AAA Lys 135	AGA Arg 135	AGA Arg 135	TTT Phe 140	CTT Leu 140	GGT Gly 140	TTT Phe 140	433
TTG Leu 145	TTA Leu 145	GGT Gly 145	GTT Val 145	GGA Gly 145	TCT Ser 145	GCA Ala 150	ATC Ile 150	GCC Ala 150	AGT Ser 150	GGC Gly 155	GTT Val 155	GCT Ala 155	GTA Val 155	TCT Ser 155	AAG Lys 155	481
GTC Val 160	CTG Leu 160	CAC His 160	CTA Leu 160	GAA Glu 160	GGG Gly 165	GAA Glu 165	GTG Val 165	AAC Asn 165	AAG Lys 165	ATC Ile 170	AAA Lys 170	AGT Ser 170	GCT Ala 170	CTA Leu 170	CTA Leu 170	529
TCC Ser 175	ACA Thr 175	AAC Asn 175	AAG Lys 175	GCT Ala 180	GTA Val 180	GTC Val 180	AGC Ser 180	TTA Leu 185	TCA Ser 185	AAT Asn 185	GGA Gly 185	GTT Val 185	AGT Ser 185	GTC Val 185	TTA Leu 185	577
ACC Thr 190	AGC Ser 190	AAA Lys 190	GTG Val 190	TTA Leu 195	GAC Asp 195	CTC Leu 195	AAA Lys 195	AAC Asn 200	TAT Tyr 200	ATA Ile 200	GAT Asp 200	AAA Lys 200	CAA Gln 200	TTG Leu 200	TTA Leu 200	625
CCT Pro 205	ATT Ile 205	GTG Val 205	AAC Asn 210	AAG Lys 210	CAA Gln 210	AGC Ser 210	TGC Cys 215	AGC Ser 215	ATA Ile 215	TCA Ser 215	AAT Asn 215	ATA Ile 215	GAA Glu 220	ACT Thr 220	GTG Val 220	673
ATA Ile 225	GAG Glu 225	TTC Phe 225	CAA Gln 225	CAA Gln 225	AAG Lys 230	AAC Asn 230	AAC Asn 230	AGA Arg 230	CTA Leu 230	CTA Leu 230	GAG Glu 235	ATT Ile 235	ACC Thr 235	AGG Arg 235	GAA Glu 235	721
TTT Phe 240	AGT Ser 240	GTT Val 240	AAT Asn 240	GCA Ala 245	GGT Gly 245	GTA Val 245	ACT Thr 245	ACA Thr 245	CCT Pro 245	GTA Val 250	AGC Ser 250	ACT Thr 250	TAC Tyr 250	ATG Met 250	TTA Leu 250	769
ACT Thr 255	AAT Asn 255	AGT Ser 255	GAA Glu 260	TTA Leu 260	TTG Leu 260	TCA Ser 260	TTA Leu 260	ATC Ile 265	AAT Asn 265	GAT Asp 265	ATG Met 265	CCT Pro 265	ATA Ile 265	ACA Thr 265	AAT Asn 265	817
GAT Asp 270	CAG Gln 270	AAA Lys 275	AAG Lys 275	TTA Leu 275	ATG Met 275	TCC Ser 275	AAC Asn 280	AAT Asn 280	GTT Val 280	CAA Gln 280	ATA Ile 280	GTT Val 280	AGA Arg 280	CAG Gln 280	CAA Gln 280	865
AGT Ser 285	TAC Tyr 285	TCT Ser 290	ATC Ile 290	ATG Met 290	TCC Ser 290	ATA Ile 295	ATA Ile 295	AAA Lys 295	GAG Glu 295	GAA Glu 295	GTC Val 295	TTA Leu 300	GCA Ala 300	TAT Tyr 300	GTA Val 300	913
GTA Val 305	CAA Gln 305	TTA Leu 305	CCA Pro 305	CTA Leu 305	TAT Tyr 310	GGT Gly 310	GTT Val 310	ATA Ile 310	GAT Asp 310	ACA Thr 315	CCC Pro 315	TGT Cys 315	TGG Trp 315	AAA Lys 315	CTA Leu 315	961
CAC His 320	ACA Thr 320	TCC Ser 320	CCT Pro 320	CTA Leu 325	TGT Cys 325	ACA Thr 325	ACC Thr 325	AAC Asn 325	ACA Thr 325	AAA Lys 330	GAA Glu 330	GGG Gly 330	TCC Ser 330	AAC Asn 330	ATC Ile 330	1009
TGT Cys 335	TTA Leu 335	ACA Thr 335	AGA Arg 335	ACT Thr 340	GAC Asp 340	AGA Arg 340	GGA Gly 340	TGG Trp 345	TAC Tyr 345	TGT Cys 345	GAC Asp 345	AAT Asn 345	GCA Ala 345	GGA Gly 345	TCA Ser 345	1057
GTA Val 350	TCT Ser 350	TTC Phe 350	TTC Phe 350	CCA Pro 355	CAA Gln 355	GCT Ala 355	GAA Glu 355	ACA Thr 360	TGT Cys 360	AAA Lys 360	GTT Val 360	CAA Gln 360	TCA Ser 360	AAT Asn 360	CGA Arg 360	1105

GTA TTT TGT GAC ACA ATG AAC AGT TTA ACA TTA CCA AGT GAA ATA AAT Val Phe Cys Asp Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn 365 370 375 380	1153
CTC TGC AAT GTT GAC ATA TTC AAC CCC AAA TAT GAT TGT AAA ATT ATG Leu Cys Asn Val Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met 385 390 395	1201
ACT TCA AAA ACA GAT GTA AGC AGC TCC GTT ATC ACA TCT CTA GGA GCC Thr Ser Lys Thr Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala 400 405 410	1249
ATT GTG TCA TGC TAT GGC AAA ACT AAA TGT ACA GCA TCC AAT AAA AAT Ile Val Ser Cys Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn 415 420 425	1297
CGT GGA ATC ATA AAG ACA TTT TCT AAC GGG TGC GAT TAT GTA TCA AAT Arg Gly Ile Ile Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn 430 435 440	1345
AAA GGG ATG GAC ACT GTG TCT GTA GGT AAC ACA TTA TAT TAT GTA AAT Lys Gly Met Asp Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn 445 450 455 460	1393
AAG CAA GAA GGT AAA AGT CTC TAT GTA AAA GGT GAA CCA ATA ATA AAT Lys Gln Glu Gly Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn 465 470 475	1441
TTC TAT GAC CCA TTA GTA TTC CCC TCT GAT GAA TTT GAT GCA TCA ATA Phe Tyr Asp Pro Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile 480 485 490	1489
TCT CAA GTC AAC GAG AAG ATT AAC CAG AGC CTA GCA TTT ATT CGT AAA Ser Gln Val Asn Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys 495 500 505	1537
TCC GAT GAA TTA TTA CAT AAT GTA AAT GCT GGT AAA TCC ACC ACA AAT Ser Asp Glu Leu Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn 510 515 520	1585
ATC ATG ATA ACT ACT ATA ATT ATA GTG ATT ATA GTA ATA TTG TTA TCA Ile Met Ile Thr Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser 525 530 535 540	1633
TTA ATT GCT GTT GGA CTG CTC TTA TAC TGT AAG GCC AGA AGC ACA CCA Leu Ile Ala Val Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro 545 550 555	1681
GTC ACA CTA AGC AAA GAT CAA CTG AGT GGT ATA AAT AAT ATT GCA TTT Val Thr Leu Ser Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe 560 565 570	1729
AGT AAC TAAATAAAAA TAGCACCTAA TCATGTTCTT ACAATGGTTT ACTATCTGCT Ser Asn	1785
CATAGACAAC CCATCTGTCA TTGGATTTTC TTAAAATCTG AACTTCATCG AACTCTCAT	1845
CTATAAACCA TCTCACTTAC ACTATTTAAG TAGATTCCTA GTTTATAGTT ATAT	1899

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 574 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr Thr Ile Leu Thr
 1             5             10             15
Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile Thr Glu Glu Phe
          20             25             30
Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala Leu
          35             40             45
Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile
          50             55             60
Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys
        65             70             75             80
Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
          85             90             95
Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
          100            105            110
Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
          115            120            125
Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
          130            135            140
Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
          145            150            155            160
Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
          165            170            175
Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
          180            185            190
Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
          195            200            205
Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
          210            215            220
Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
          225            230            235            240
Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
          245            250            255
Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys
          260            265            270
Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile
          275            280            285

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Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro
 290                               295                               300

Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro
305                               310                               315                               320

Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg
                               325                               330                               335

Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
                               340                               345                               350

Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp
                               355                               360                               365

Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn Leu Cys Asn Val
 370                               375                               380

Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr
385                               390                               395                               400

Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys
                               405                               410                               415

Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile
                               420                               425                               430

Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Met Asp
                               435                               440                               445

Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly
                               450                               455                               460

Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro
465                               470                               475                               480

Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn
                               485                               490                               495

Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu
                               500                               505                               510

Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile Thr
                               515                               520                               525

Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala Val
                               530                               535                               540

Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu Ser
545                               550                               555                               560

Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe Ser Asn
                               565                               570

```

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
1				5					10					15

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/13694

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395, 39/42; C12Q 1/00, 1/70; G01N 33/53

US CL : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,811,524 A (BRAMS et al) 22 September 1998, cols. 12- 20.	1, 4, 10-15 ----- 2, 3
X ----- Y	US 5,824,307 A (JOHNSON) 20 October 1998, cols. 4-6.	1, 4, 10-15 ----- 2, 3
X ----- Y	US 5,880,104 A (LI et al) 09 March 1999, cols. 6-10.	1, 4, 10-15 ----- 2, 3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 AUGUST 2000

Date of mailing of the international search report

05 SEP 2000

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/13694

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, DIALOG, MEDLINE

search terms: RSV, respiratory syncytial, monoclonal, antibodies, human, humanized, F protein, diagnostics, passive immunization, therapy, treatment